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14. ABSTRACT The Vietnam Head Injury Study - Phase III (VHIS-P3) experienced contractual and logistical delays early on in the project, but by April 2004 all contracts, space allocation, and IRB approvals had been obtained. Participant evaluations commenced on 27 Apr 2004 and ceased on 31 Oct 2006. A total of 199 patients and 55 controls (23 were newly recruited) were enrolled. There were 13 adverse events, none of which were related to the study. Computed Tomography (CT) scans were performed on 198 subjects and electroencephalographs (EEG) on 171. To date, 100 EEG and all CT reports have been received. Blood samples were collected on 252 participants for genetic analysis and 253 participants agreed to be videotaped. The National Death Index has provided information on causes of death for all Phase 2 and 3 VHIS registrants who have died and that database has been established. All participant data has been collected and entered into the VHIS master database; containing over 4500 data points per participant. All collaborators have received data sets for analysis and are currently either performing analyses or preparing manuscripts. All data has been copied and transferred to the NIH for archiving. Several presentations and manuscripts have already resulted from preliminary analyses and many more are in progress.					
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INTRODUCTION

This report summarizes events for the entire award period of 24 Sep 2001 through 23 Aug 2007 in the implementation of the Vietnam Head Injury Study - Phase III: A 30-Year Post-Injury Follow-up Study, funded by the US Army Medical Research and Material Command, under grant no. DAMD17-01-1-0675.

BODY

Summary:

The Vietnam Head Injury Study - Phase III (VHIS-P3) experienced contractual and logistical delays early on in the project, but by April 2004 all contracts, space allocation, and IRB approvals had been obtained. Participant evaluations commenced on 27 Apr 2004 and ceased on 31 Oct 2006. A total of 199 patients (182 of whom participated in the Phase 2) and 55 controls (32 of whom had participated in the Phase 2; 23 were newly recruited) were enrolled. There were 13 adverse events, none of which were related to the study. Computed Tomography (CT) scans were performed on 198 subjects and electroencephalographs (EEG) on 171. To date, 100 EEG and all CT reports have been received. Blood samples were collected on 252 participants for genetic analysis and 253 participants agreed to be videotaped. The National Death Index has provided information on causes of death for all Phase 2 and 3 VHIS registrants who have died and that database has been established. All participant data has been collected and entered into the VHIS master database; containing over 4500 data points per participant. Furthermore, all collaborators have received data sets for analysis and are currently either performing analyses or preparing manuscripts. All data has been copied and transferred to the NIH for archiving. Several presentations and manuscripts have already resulted from preliminary analyses (see reportable outcomes section, referenced below and full copies enclosed as appendices) and many more are in progress.

Statement Of Work review:

In November 2002, the study coordinator for the project was selected, and began working on 25 Nov 2002. At that point, recruitment advertisements for the remaining needed staff were placed in appropriate journals and newsletters and interviews commenced.

The protocol to study VHIS-P3 subjects at National Naval Medical Center (NNMC) was submitted to their Institutional Review Board (IRB) in March 2003. Approval from the NNMC IRB was granted in May 2003; however, NNMC mandated a Scientific Peer Review by the National Institute of Neurological Disorders and Stroke (NINDS). Peer approval was granted in August 2003. The NINDS IRB then entered into an agreement with the NNMC IRB in early September to rely upon the NNMC IRB for the protection of human subjects. Subsequently, the protocol was delivered to USAMRAA for their review and approval on 17 Sep 2003.

In June 2003, the NNMC Department of Radiology confirmed their capability to perform the VHIS-P3 scans at study specifications. The Walter Reed Army Medical Center (WRAMC), site of the VHIS- Phase 2, agreed to transfer the Phase 2 CT scans for use (comparison) by the NNMC Department of Radiology. In October 2003, a Memorandum of Understanding (MOU) was executed with WRAMC to obtain the VHIS- Phase 2 CT brain scans for all subjects.

After receiving permission from the Vice Acting Chair of the Human Subjects Research Review Board (HSRRB) to contact the 520 subjects who participated in the 1981-85 study (Phase 2) for confirmation of contact information, the Veterans Administration (VA) Privacy Division forwarded the NNMC approved VHIS-P3 Confirmation of Information Request. All letters were sent to the Phase 2 subjects and controls from 8 Aug 2003 to 15 Sep 2003. Three hundred twenty head-injured subjects expressed interest in attending Phase 3 of the study. Of the original 85 control subjects without head injuries, 31 responded positively. At that time, it was decided that further control subjects would be recruited through veteran publications.

In May 2004, the control recruitment advertisement received approval by NNMC, and a website was created to publicize the VHIS. In June a press release was issued to 23 media outlets. The study was publicized in the “Pentagram” and in the NNMC base newspaper “The Journal”, and in July in the Washington Times. In September the American Legion Magazine published the control recruitment advertisement, from which we received a positive responses from dozens of potential control subjects.

By November 2003 all study staff had been hired and testing equipment (hardware, software, supplies) and office machines were purchased. All mechanisms for transportation, lodging, and meals to be provided to VHIS-P3 participants were established. The complete testing battery was formalized and research assistants were trained to provide support for the participants, administer and score all tests, and enter data into a format for the database.

In January 2004, non-Department of Defense beneficiary participants were granted Secretary of the Navy Designee Status to cover medical care associated with participation in the VHIS study. At this time NNMC also approved the Phase 3 consent forms. We received final Command approval on 24 March 2004, and began enrolling subjects on 27 Apr 2004. We initially saw increasing numbers of subjects to test the tolerability of the battery, commencing with one, then two and then three subjects every 7-8 days.

From 2 Jun 2004 we continued to test three subjects approximately every 7-10 days, with the battery taking 7 full days for each subject to complete. Data began to be entered into the VHIS – P3 computer database on a continual basis. In July 2004 final adjustments were made to the Analysis of Brain Lesions (ABLE) computer program to allow comprehensive examination of the CT brain scans taken in Phase 3. In September 2004 the testing battery was reduced to 5 days, due to problems with subject fatigue. Roles of individual study staff were reviewed to afford the most time-effective schedule for subject testing.

During July 2004 a modified Statement of Work (SOW) was submitted and approved by USAMRMC. In October 2004 we received NNMC approval of modified consent forms and HIPAA documents due to a change in the NNMC Principal Investigator from Dr. George McKenna to Dr. John Hughes.

In February 2005 we completed our NNMC Annual review. In March 2005 we received continued approval from our audit, and there was a NNMC approved modification of the protocol.

At this point we had identified two cases of probable undetected epilepsy and two further cases with likely meningiomas.

In September 2005 we ran an advertisement in the Disabled American Veterans publication for further control recruitment, with a number of positive responses. The following month, with the continued assistance of the VA compensation division, we sent out a mailing inviting subjects identified in the original VHIS Registry, but who did not attend Phase 2 of the study, to come to the NNMCMC for assessment.

In January 2006 we underwent the NNMCMC annual review and the following month the study was endorsed as having minimal risk and was approved for another year. In March an amendment to add information from the National Death Index (NDI) was submitted to the NNMCMC IRB and it was approved the following month.

All brain-injured patients who indicated an interest in participating were scheduled. The final subject evaluation ended on 31 Oct 2006.

In late December 2006, the VHIS-P3 petitioned the NNMCMC IRB for permission to copy all VHIS records and store them at the NIH under the protection of Dr. Grafman. Furthermore, we asked for deferral of the NNMCMC publication process to be cleared solely by the NIH. On 9 Jan 07, we were granted approval for both requests.

In January 2007, we underwent our continuing annual review and once again received endorsement at Minimal Risk with an extension to March 2008.

In April of 2007, due to a change in duty station, Dr. John Hughes was removed as the NNMCMC Principal Investigator and Dr. William Watson was added. IRB approval was quickly forthcoming. Later that month, we received a no cost extension from the original closing date of 23 May 2007 to 23 Aug 2007 from the USAMRMC to complete accurate recording, disseminating and storing the wealth of data collected by the VHIS.

A bill for the completed CT scans, EEGs, and costs associated with occupying offices was forthcoming from the NNMCMC Resource Management Directorate and is currently being processed by the Congressional Program Services Office of Sponsored Programs at the Henry M. Jackson Foundation for the Advancement of Military Medicine (HJF).

The National Death Index has provided information on causes of death for all Phase 2 and 3 VHIS registrants and that database has been established. All participant data has been collected, verified and entered into the VHIS master database; containing over 4500 data points per participant.

We have commenced statistical analysis on the entire data set and manuscript writing has begun. Currently, Dr. Vanessa Raymont and colleagues are about to submit a manuscript to the journal *Brain* entitled, "Demographic, Structural and Genetic Predictors of Late Cognitive Decline After Penetrating Head Injury" (see appendix A), and Dr. Michael Koenigs and colleagues are preparing the manuscript, "Focal brain damage protects against post-traumatic stress disorder in combat veterans" for submission to the journal *Nature* (see appendix B).

All collaborators have received copies of their data sets and have begun analysis. All data has been copied to CD/DVD and will be transported to NIH for safe keeping by Dr. Grafman. A final report has been submitted to the NNMCMC IRB and forwarded to the Human Subjects Protection Department at USAMRMC. As of 23 Aug 2007, all property and fixed assets will be returned or donated to the appropriate sources and the remaining staff members, reported below, will disperse.

KEY RESEARCH ACCOMPLISHMENTS/SUMMARY OF WORK

- CT scans performed: 198
- EEGs performed: 171, reports received for 100
- Blood Samples collected: 252
- Videotapes recorded: 253
- Total participants seen: 254
 - Subjects: 199
 - Registrants returning from Phase 2: 182
 - Registrants who did not participate in Phase 2: 17
 - Controls: 55
 - Returning from Phase 2: 32
 - Newly recruited: 23
- Two subjects and no controls have died since their participation in the VHIS phase 3.
- Total Adverse Events reported: 13 – none related to study.

REPORTABLE OUTCOMES

Manuscripts (see appendices for full copies):

1. Raymont V, Greathouse A, Reding K, Lipsky R, Salazar A, Grafman J. Demographic, Structural and Genetic Predictors of Late Cognitive Decline After Penetrating Head Injury. In Preparation.
2. Koenigs M, Huey E, Raymont V, Cheon B, Solomon J, Wassermann E, Grafman J. Focal brain damage protects against post-traumatic stress disorder in combat veterans. In Preparation.

Presentations:

1. Coelho C. Analysis of brain lesions associated with narrative discourse impairments following penetrating head injury. Clinical Aphasiology Conference 2006, May 29-June 2, Gent, Belgium.
2. Ngo CT. Neural Substrates of Semantic Memory Learning and Retrieval. Baylor College of Medicine, Houston, TX. November 15, 2006.
3. Ngo CT. Neural Substrates of Semantic Memory Learning and Retrieval. The George Washington University, Washington, DC, January 2007.
4. Swanson E, Raymont V, Grafman J, Salazar A, Reding K. Prevalence and Associations of Post Traumatic Epilepsy in the VHIS. 12th Annual GWUMC Health Sciences Research Day, March 26, 2007, Washington, DC.
5. Ngo, CT. "Neural Substrates and Mechanisms Underlying Semantic Fluency Deficit in Traumatic Brain Injured Adults". Ph.D. dissertation, The George Washington University, 2007.

6. Grafman, J. “Long Term Outcome in the Vietnam Head Injury Study.” Institute of Medicine, National Academy of Sciences, August 9th, 2007, Washington, D.C.

Remaining Staff:

Sandra Bonifant – Study Coordinator

Katherine Reding – Data Manager

CONCLUSION

As the two about-to-be submitted studies indicate, we anticipate learning novel information from the VHIS – Phase 3 that has significant impact on both the care of head injured veterans as well as improving knowledge about basic brain-behavior relations. For example, in the first two manuscripts in preparation, we have found:

- Those with penetrating head injuries (PHI) demonstrated a greater degree of cognitive decline as compared to a control group of uninjured Vietnam veterans and this became increasingly significant later in life. We also found that preinjury intelligence was the most consistent predictor of cognitive outcome and that there is evidence for an association between level of cognitive decline following PHI and the possession of certain genetic markers that have been linked with brain injury and neurodegeneration. These findings are important for both planning for the clinical care of patients with brain injuries but also for our understanding of neuroplasticity.
- We discovered a reduced occurrence of post traumatic stress disorder (PTSD) following ventromedial prefrontal cortex (vmPFC) damage and the complete absence of PTSD following amygdala damage. These results show that vmPFC and amygdala are critically involved in the pathogenesis of PTSD, and suggest that interventions aimed at selectively disrupting vmPFC and/or amygdala function could have efficacy to treat PTSD. These findings are important for both deciding upon how to triage patients with brain injury and exposed to stressful circumstances as well as identifying the key brain areas important for the development of PTSD.

Both these papers, we hope, signify the beginning of a series of papers that increase our basic understanding of the long term effects of head injury on cognitive, social and neurological functioning (as the VHIS Phase 2 did), but also set the bar for clinical studies of war wounded US veterans with brain injuries. We hope the knowledge that we will impart from these studies help future planners in taking care of our brain-injured veterans and improving their treatment. We are grateful for the opportunity.

APPENDIX A

DEMOGRAPHIC, STRUCTURAL AND GENETIC PREDICTORS **OF LATE COGNITIVE DECLINE AFTER PENETRATING HEAD INJURY**

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The views expressed in this article are those of the author and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, nor the U.S. Government.

ABSTRACT

We examined the relationship of preinjury intelligence, brain tissue volume loss, lesion location, demographic variables and a number of genetic markers to long-term cognitive decline in a group of Vietnam veterans with predominantly penetrating head injury (PHI) suffered more than thirty years ago. Using linear and stepwise regression procedures, we found that those with PHI demonstrated a greater degree of cognitive decline overall during the years following injury compared to a control group of uninjured Vietnam veterans. This became increasingly significant later in life. We also found that preinjury intelligence was the most consistent predictor of cognitive outcome across all phases of potential recovery and decline after such injuries. Laterality of lesion was not a factor. Finally, we found evidence for an association between level of cognitive decline following penetrating head injury and the possession of certain genetic markers that have been linked with brain injury and neurodegeneration. Thus exacerbated decline does occur in Vietnam veterans with PHI, is apparently unrelated to dementia and is determined by multiple factors (most notably preinjury intelligence).

Key words: Cognitive decline, brain injury, penetrating brain injury, genetics, predictors.

Total number of words: 8,921

This study aimed to investigate the associations between long term indicators of general intelligence and penetrating head injury (PHI). This has been an area of limited study to date, with the great majority of research involving closed head injuries. We examined the

relationship between preinjury intelligence, brain volume loss and late cognitive decline 36-39 years post injury, in a group of Vietnam veterans with PHI. We also investigated whether site of injury within the brain or degree of tissue loss with aging may affect levels of general intelligence decades after a PHI, and if certain genetic polymorphisms may influence long term cognitive outcome after penetrating head injury.

IMPORTANCE OF TRAUMATIC BRAIN INJURY

Traumatic brain injury (TBI) is the primary cause of death and disability in those under 35 in the U.S., with civilian penetrating head injuries (PHI) being one of the fastest-growing types of head injury. Each year approximately 55,000 deaths result from TBI and an additional 50,000 people suffer from persistent physical, cognitive, behavioral, and social deficits resulting from TBI (Kraus, 1996). TBI remains prevalent in combat situations, with nearly two-thirds of injured U.S. soldiers sent from Iraq to Walter Reed Army Medical Center having been diagnosed with traumatic brain injuries. Of the 58,000 U.S. combat fatalities in the Vietnam war, about 40% were due to head and neck wounds. Overall, about 19% of casualties and 14% of survivors suffered a head injury. Early field care and rapid helicopter evacuation, combined with the deployment of neurosurgical teams close to the battlefield resulted in survival of many more severely wounded men than in previous conflicts (Rish *et al.*, 1983; Hammond, 1986; Carey, 1987).

The military population offers a number of advantages for the study of the long-term effects of head injury: its size, relative uniformity, and the potential for long-term follow-up. Also, young recruits were, by definition, healthy and employed preinjury, and preinjury intelligence and aptitude testing is available on most of them for comparison with post injury performance. Additionally, the Veterans Affairs (VA) medical system has allowed them to be tracked over a long follow-up period. Finally, the low-velocity

penetrating fragment wounds typically sustained at the time of Vietnam resulted in relatively focal defects, which allow for unique brain structure-function studies. Thus these patients, in particular, can provide unique information regarding the effects of PHI on long-term cognitive and social functioning.

CONNECTIONS BETWEEN TBI AND COGNITIVE DECLINE

The possibility of cognitive decline many years following head injury has been increasingly discussed in recent times (Brooks, 2003), though it remains poorly understood (Himanen, 2006). Any progress has been hindered by the lack of studies able to examine relatively discrete brain lesions, as little research has been conducted with penetrating brain injuries (Corkin *et al.*, 1989; Grafman *et al.*, 1988). Additionally, most analyses have focused on the link between head injury and dementia, not on the concept of a unique process of accelerated cognitive decline specific to TBI.

In one of the few studies that have examined the effects of focal and penetrating head injuries on long term cognitive deterioration, Corkin *et al.* (1989) examined PHI survivors from the second world war and found that head injury did indeed exacerbate the cognitive decline of normal aging, with left hemisphere injuries having a greater impact than right hemisphere lesions. There appeared to be some site-specific effects, with subjects with left posterior lesions showing greatest decline on verbal-based cognitive testing (including vocabulary and arithmetic), and those with right parietal lesions showing exacerbated decline on tests of spatial functioning. Additionally, while subjects with left parietal lobe damage showed decline in the greatest number of neuropsychological subtests, those with frontal lobe lesions displayed no increased level of cognitive decline. While the numbers were too small to make any definitive conclusions, this study laid the groundwork for future studies of penetrating head injuries.

More recently, Himanen also demonstrated a decline in most cognitive domains many years after head injury. Subjects showed greater deterioration on the performance subtests of the WAIS compared to the verbal subtests (Himanen, 2006). Those injured in the second or third decade of life showed greater improvement than other head-injured individuals, however they still performed at a lower level than controls on all cognitive tasks. Subjects with more severe injuries showed particularly high levels of decline in verbal learning. Some studies have also shown a link between mild TBI and accentuated 'normal aging', with evidence that even minor CHI leads to earlier onset and accelerated cognitive aging (Klein *et al.*, 1996).

These data have led to the development of the 'margin of safety model' of the long term effects of head injury (Corkin *et al.*, 1989). This relates to the repeated observation that there is not a direct relationship between the degree of brain pathology or damage and the clinical manifestation of that damage (Stern, 2006). There are two proposed explanations for this; the concept of 'brain reserve' or 'threshold model' (Satz, 1993), which suggests that reserve derives from more richly intra- and inter-connected neuronal networks, so that deficits only occur when brain reserve is depleted beyond a threshold. The second is the 'cognitive reserve model', which suggests that the brain attempts to cope with any damage by utilizing either preexisting networks in a more efficient manner (the 'neural reserve' theory) or by recruiting alternative networks ('neural compensation'), although it is also suggested that the models do not operate in mutual exclusivity (Stern, 2006). Educational attainment has been postulated as a marker for cognitive reserve, although it is likely to be supplemented by genetics, physical conditioning and later life experiences. One prospective study showed that general intelligence in the fifth decade was separately influenced by childhood cognitive abilities, education and occupation in adulthood (Richards and Sacker, 2003). The idea that cognitive or neuronal reserve may delay the onset of clinically relevant cognitive and functional impairment has been proposed as a

way to explain the consistent observation of a lower risk of dementia among intelligent and well educated people (Cervilla *et al.*, 2004). Some studies, however, have suggested that those with a greater cognitive reserve have a more rapid decline once a dementia is detected (Stern *et al.*, 1995).

The link between TBI and increased risk of developing dementia later in life, however, remains ambiguous (Mehta *et al.*, 1999; Fleminger *et al.*, 2003; Millar *et al.*, 2003; Mayeux, 1996; Newcombe, 1996). Some research has suggested that the risk of developing dementia increases as the severity of the injury increases (Plassman *et al.*, 2000; Rapoport *et al.*, 2004; Mehta *et al.*, 1999; Mortimer *et al.*, 1991; Himanen, 2006), while other studies have failed to show any increased risk of dementia in following CHI (Mayeux, 1996). In one meta analysis, Fleminger *et al.* (2003) found no significant association between head injury and Alzheimer's disease (AD) in seven studies. A recent hypothesis is that head injury may merely lead to earlier onset of dementia, rather than increasing the lifetime risk of developing the disease (Rapoport *et al.*, 2004; Mehta *et al.*, 1999).

GENETIC ASSOCIATIONS WITH COGNITIVE DECLINE

It has been established that most neurodegenerative processes results from complex interactions between both environmental effects and genetic factors (Lindsay *et al.*, 2002). In recent years, there has been increasing evidence of links of between specific genotypes and risk for accelerated cognitive decline or dementia following CHI. Below we briefly touch on the data from a few selected genotypes that we examined in this study.

The genetic association between Apolipoprotein E [epsilon]4 (APO e4) and late-onset AD was first reported in 1993 (Strittmatter *et al.*, 1993; Corder *et al.*, 1993), and has since been confirmed by many studies (Mayeux *et al.*, 1993a). Several groups have also found that APO e4 is a risk factor for poor outcome after moderate to severe CHI (Teasdale *et al.*, 1997; Mayeux *et al.*, 1993b, 1995; Friedman *et al.*, 1999; Plassman *et al.*, 2000). Although the mechanisms underlying these effects are unclear, some evidence suggests that both APO e4 and CHI may influence the risk of AD via interactions with the amyloid- β (A β) peptide. A β deposition can be found in approximately 30% of people who die shortly after CHI (Roberts *et al.*, 1991), and a significant percentage of these patients are APO e4-positive (Nicoll *et al.*, 1995, 1996; Teasdale *et al.*, 1997; Lichtman *et al.*, 2000).

It is not clear from the available data at what point in the course of CHI APO e4 has its primary effect. Several reports find that APO e4-positive individuals are more likely to have a poor presentation (Teasdale *et al.*, 1997; Friedman *et al.*, 1999; Millar *et al.*, 2003; Jiang *et al.*, 2006) and lower early cognitive function (Lieberman *et al.*, 2002; Millar *et al.*, 2003; Ariza *et al.*, 2006). Intraneuronal APO e is markedly increased after acute TBI; thus, APO e is also a candidate for modified outcome after TBI, possibly because it is involved in the response to neural injury and repair (Laskowitz *et al.*, 1998). Other studies suggest (Lichtman *et al.*, 2000; Crawford *et al.*, 2002) that the APO e4 effect persists after controlling for severity factors, implying that it may play a role in neural repair and regeneration. Isoniemi *et al.* (2006) found no association between hippocampal volumes, lateral ventricle volume, and APO e4 several decades after CHI. Hence, if the APO e4 allele is associated with an unfavorable outcome after traumatic brain injury, this association may involve mechanisms other than those responsible for the development of normal brain atrophy.

Inheritance of APO e4 may also influence cognitive dysfunction that is related to TBI but delayed by years or even decades after the injury (Starkstein and Jorge, 2005). Several case-control studies indicate that possession of APO e4 together with head injury increases the risk of developing AD in later life (Mortimer *et al.*, 1985, 1992; Graves *et al.*, 1990; Mayeux *et al.*, 1993; Plassman *et al.*, 2000; Lendon *et al.*, 2003), although several investigators were unable to verify these findings (Chandra *et al.*, 1989; Salib and Hillier 1997; O'Meara *et al.*, 1997; Mehta *et al.*, 1999; Millar *et al.*, 2003). A recently published meta-analysis of 15 studies published from 1985 to 1995 (Fleminger *et al.*, 2003) found an elevated risk of AD (odds ratio = 1.58, 95% confidence interval = 1.21–2.06) for males with a history of brain injury, but not for females. Since the increased risk of AD after CHI appears to be influenced by family history of AD (Mayeux *et al.*, 1993b), Mayeux *et al.* (1995) studied the synergistic effect of head injury and inheritance of the APO e4 allele. They found that while APO e4 increased the risk of AD 2-fold, the occurrence of CHI in APO e4-positive individuals increased the risk of AD 10-fold. There was no increased risk of AD in subjects who suffered brain injury but were APO e4 negative. Thus these risk factors appear to act synergistically, in that individuals who are APO e4-positive are even more likely to develop dementia if they sustain CHI at some time in their life (Tang *et al.*, 1996). We were interested in whether APO e4 was associated with change in intelligence, either in the initial recovery period or in the subsequent years of possible decline.

COMT

The catechol-O-methyltransferase (COMT) gene is essential for the metabolic degradation of dopamine in the prefrontal cortex. A single nucleotide polymorphism leading to a Val to Met substitution (Val¹⁵⁸Met) in the coding region of the COMT gene appears to influence activity levels of the enzyme, with the Met allele having one quarter of the activity of the Val allele (Lachman *et al.*, 1996). Hence individuals with the

Met/Met genotype have been found to display better prefrontal functioning than the Met/Val or Val/Val genotypes (Egan *et al.*, 2001; Malhotra *et al.*, 2002; Mayer-Lindenberg, 2005; Gothelf *et al.*, 2005). COMT has been linked to variability in working, episodic and semantic memory, and age-related deficits in cognitive functioning appear to be at least in part affected by the availability of dopamine (Bäckman and Farde, 2004; Stefanis *et al.*, 2005). Hence COMT has been suggested as a candidate for genetic predictability of cognitive decline in aging (de Frias *et al.*, 2005). In a study of 292 men (without any diagnosed dementia) over 5 years, de Frias and colleagues (2005) found those with the Val COMT allele performed worse on tests of working memory, executive function and visuospatial ability.

Similar results have been found in those with TBI. Lipsky *et al.* (2005) found subjects with a history of TBI that were homozygotes for the low enzyme activity polymorphism (COMT Met) performed better on tests of executive functioning than individuals with the high enzyme activity polymorphism. In our study we hypothesized that the presence of a COMT polymorphism may dictate performance on general cognitive abilities, and in particular that those with the Met/Met genotype may have a greater level of protection against cognitive decline after PHI.

GRIN

Experimental animal studies have revealed impaired plasticity following TBI, even in the absence of significant anatomical damage (Giza, Maria and Hovda, 2006), evidenced by examining N-Methyl-d-aspartate (NMDA). NMDA consists of a number of subunits, including the GRIN glutamate receptor, which seems to be specifically involved in the pathophysiology of CHI. Previous studies have suggested an increase in glutamate following CHI triggers neuronal death, and may be important in subsequent cognitive

deficits (Parton, Coulthard and Husain, 2005). Thus it is feasible that GRIN genotype may influence initial responses to PHI.

BDNF

Brain-derived neurotrophic factor (BDNF) is an endogenous protein involved in the maintenance of neuronal function, synaptic plasticity and structural integrity of the adult brain. Levels of BDNF in the brain have been found to correlate with severity of cognitive decline in AD (Chuu *et al.*, 2006; Laske *et al.*, 2006). In fact, upregulation of trophic factors, such as BDNF, via motor exercise, may prime the brain to respond more favorably to injury, inoculating against further damage and enabling recovery and local compensation (Kleim, Jones and Schallert, 2003). Thus they reflect endogenous attempts at neuroprotection, and so maybe an early marker of brain injury (Chiaretti *et al.*, 2003). Therefore we hypothesized that BDNF may have a role in both early and long term plasticity following PHI.

DBH

Studies have suggested for some time that dopamine plays a role in CNS plasticity after brain injury (Clifton, Ziegler and Grossman, 1981), reflected in a reduction in dopamine beta-hydroxylase (DBH) immunoreactivity (Zhu *et al.*, 2000). Conversely, DBH has been found to be significantly raised in those with AD (Giubilei *et al.*, 2004). It certainly appears that DBH has some role in facilitating cognitive recovery after brain injury (Zhu *et al.*, 2000). We were especially interested in whether DBH played a role in degree of exacerbated decline during the later period of recovery from PHI.

GAD

Glutamic acid decarboxylase (GAD) is the rate-limiting enzyme for the production of gamma-amino butyric acid (GABA) in the brain. The adenosine triphosphate (ATP)-mediated control of GABA synthesis gradually declines with age and AD-related neurodegeneration (Marczynski, 1998). Additionally, GAD has been associated with short-term plasticity (Ramsey *et al.*, 2004), and the neurological deficits resulting from brain injury-induced white matter lesions (Robinson *et al.*, 2006). In animals, brain injury has been found to increase levels of GAD, causing inhibition of prefrontal neuronal activity (Kobori and Dash, 2006). Therefore it is feasible that the periods both early on and many years after a PHI may be influenced by the presence or absence of GAD genotypes.

Thus it seems likely that besides the important effects of our patients' preinjury cognitive development, the initial neural response to PHI and any subsequent plasticity or neurodegeneration processes are all heavily influenced by genetic factors (Lindsay *et al.*, 2002), some of which may have important roles at differing times post-injury. We hypothesized that GRIN may, in particular, play an important role in the early response to neural damage in PHI. In addition it seemed plausible that APO e4, BDNF, DBH and GAD genotypes may have an effect at a number of time points during the period after PHI.

BACKGROUND AND PREVIOUS RELEVANT FINDINGS OF THE VIETNAM HEAD INJURY STUDY

The Vietnam Head Injury Study (VHIS) is a prospective, long-term follow-up study of

head-injured Vietnam veterans. The subject registry was collated during the Vietnam conflict by Dr. William Caveness at the National Institutes of Health. Simple registry forms outlining demographic, injury and initial outcome data were completed by military physicians in Vietnam on head injured soldiers who had survived the first week after a severe head injury. About 2,000 patients were entered in the registry between 1967 and 1970. Phase 1 (P1) of the VHIS was a medical records review some 5 years post-injury using the military and VA medical and personnel records of 1,221 of these men, for whom adequate field, hospital, rehabilitation, and follow-up records were available.

Phase 2 (P2) was a collaborative project of the three Military Services; the Department of Veterans Affairs, the National Institutes of Health, and the American Red Cross. It consisted of a comprehensive, multidisciplinary inpatient evaluation at Walter Reed Army Medical Center. Approximately 520 head injured subjects from the original registry and 85 matched normal volunteers were evaluated between 1981 and 1984, some 12-15 years post injury. Many of these patients had been lost to medical follow-up and were not receiving medical services or had inappropriately received less than honorable discharges for behavioral changes related to their brain injuries. Phase 2 thus also served to identify these patients and refer them for appropriate care, or correct their military records. Of the 520 patients, seventy-seven (77%) percent had missile fragment wounds, 15% had gunshot wounds, and only 8 % had a closed head injury (CHI). Seventy-eight percent had multiple lobe injuries and 30% had bilateral lesions. When the impact of education, preinjury intelligence, brain volume loss, and lesion location on postinjury intelligence level was examined, it was found that in general, the most important determinant of postinjury intelligence was preinjury performance as assessed by the Armed Forces Qualification Test (AFQT; Grafman *et al.*, 1988). In addition, the more global the cognitive test, the greater the effect of brain loss volume, with specific cognitive processes being affected relatively more by lesion location (Grafman *et al.*,

1986).

DIFFICULTIES WITH EXISTING RESEARCH DATA

There have been a number of difficulties inherent in the methodology of previous studies of the long term cognitive outcome following TBI. Firstly, there have been a wide variety of definitions of head injury employed and inconsistency in the standard scale used for assessing the severity of the head injuries. Additionally, the concept of what represents exacerbated or accelerated cognitive decline has yet to be fully defined, which may also lead to an increased possibility of the misdiagnosis of dementia in some subjects (Fleminger *et al.*, 2003). What represents an abnormal pattern of decline also raises questions regarding what time period post-TBI may be associated with the greatest risk for decline. There is some evidence that motor, sensory, and cognitive functions seemed to improve in the first few years after a TBI and then reach a plateau (Walker, 1989), but whether the level of initial recovery has any association with subsequent decline remains undetermined.

Regarding the study designs, many have involved small numbers of participants with head injuries, leading to a potential lack of statistical power necessary to detect any associations (Fleminger *et al.*, 2003). There remain important limitations in the largely case-control genetic studies, primarily related to recall bias and the lack of access to complete medical records regarding the TBI (Diaz-Arrastia and Baxter, 2006). Most studies have also focused on subjects with CHI, whereas combat head injury in past conflicts typically involved many more penetrating lesions. Finally, a number of studies have recognized the difficulty in accounting for other factors that may contribute to long term cognitive impairment, such as alcohol use and cardiovascular disease (Klein *et al.*, 1996; Walker, 1989).

AIMS OF THIS STUDY

Largely by virtue of the unique focal nature of their injuries, and their comparable personal status at the time of injury, the VHIS population can provide novel insights into a number of questions relating to brain function and recovery from TBI. We aimed to address the link between general demographic factors, such as educational level and race, as well as the site and size of PHI, and long term cognitive outcome as measured by an intelligence score surrogate. We also wanted to examine the possible impact of genetic polymorphisms on neuroplasticity in the aging, damaged brain and whether they play a part in predicting exacerbated cognitive decline or onset of dementia.

METHODOLOGY

Subjects

The subjects were drawn from the Vietnam Head Injury Study registry, 92% of whom had a history of a penetrating head injury. Phase 3 (P3) has been modeled upon the Phase 2 (P2) Vietnam Head Injury Study. Of the 520 head-injured subjects that were assessed in Phase 2, 484 are still alive, and 182 attended Phase 3 of the study. Additionally, 17 patients identified in P1 but that did not attend P2 were assessed. Of the original 80 control subjects without head injuries recruited in P2, 32 attended P3 and a further 23 were recruited for P3, through advertisements in veteran publications. Subjects were assessed over 5-7 days at the National Naval Medical Center in Bethesda, Maryland.

There were no significant differences between the head-injured and control subjects in terms of age at P3 testing, total years of education or induction intelligence level (as measured by the Armed Forces Qualification Test; AFQT) (Table 1). 186 were right-handed (144 head-injured, 42 controls) and 28 were left-handed (20 head-injured, 8 controls). In addition, 13 of the head-injured group were originally right handed but now are forcibly left handed (because of hemiparesis), and 6 were originally left handed but now are forcibly right handed. Six of the head-injured group and three control subjects described themselves as ambidextrous.

-----TABLE 1 ABOUT HERE-----

A t-test was run to compare those who underwent assessment only in P2 (338 head-injured and 48 controls), compared to those who attended both P2 and P3. Age (at start of P3 testing), total years of education, preinjury AFQT, P2 AFQT score, and total lesion volume loss and laterality were compared between attenders and non-attenders of P3. Comparing the whole group (head-injured and controls), there was no significant differences in age between P3 attenders and non-attenders. However, those that attended P3 had more years of education ($t=-3.062$, $df=601$, $p=0.002$), and a higher AFQT score both preinjury ($t=-4.851$, $df=581$, $p<0.001$) and at P2 ($t=-6.151$, $df=571$, $p<0.001$), than P3 non-attendeers. This was also the case when the head-injured group was assessed alone, but not within the control group. Within the controls, there were no significant differences in educational attainment, preinjury AFQT, or P2 AFQT between those who did and did not attend P3.

CT scan analysis

Brain lesions were identified by CT scan, and the data were reconstructed with a 3mm overlapping slice thickness and a 1mm interval. Lesions were processed using ABLe software ('Analysis of Brain Lesions'; Makale *et al.*, 2002; Solomon *et al.*, 2007). ABLe is an interactive program run via MedX medical imaging software (Medical Numerics Inc., Sterling, VA), that determines the lesion size and cytoarchitectonic brain regions contained within the lesions space. Within ABLe, the lesions were drawn manually in native space on each 1mm thick slice by VR (a psychiatrist with clinical experience of reading CT scans), and reviewed by JG, enabling a consensus decision to be reached regarding the limits of each lesion. Lesion volume was calculated and the brain images automatically registered to a template brain in Talairach space (Talairach and Tournoux, 1988). The template image we used was derived from a CT scan of a 27-year-old male, conformed to Talairach dimensions in MedX using an affine 12-parameter transformation derived from the Automated Image Registration (AIR) software within MEDx (Makale *et al.*, 2002). Computerized graphics of Brodmann areas were derived by mapping onto a resliced version of the MRI image. Thus the intersection of lesions with Brodmann areas could be determined using the VOTL database with in ABLe, as could the coordinates comparable to the templates produced by Damasio and Damasio (1989). This procedure allowed the measurement of normalized lesion volume and percentage of brain regions involved. We used the difference in the lesion volume calculated in P2 and P3 to give one estimated measurement of atrophic change over time. In addition three other measurements of atrophy were made via a consensus decision between a trained neurologist (AS) and VR from P3 CT scans: corpus callosum width (millimeters), and a rating of global brain atrophy and specific areas of atrophy (both on a scale of from 0 to 7). Third ventricle width had previously been shown to correlate well with other measures of brain atrophy on CT scans (Reider-Groswasser *et al.*, 2002). An analysis was carried out to assess the correlation between these measures. Third ventricle width correlated significantly with the corpus callosum width measurements ($r=0.416$, $p<0.001$;

$r=0.296$, $p=0.001$; $r=0.352$, $p<0.001$), as well as the global assessment of atrophy ($r=0.377$, $p<0.001$).

Tests

Subjects were assessed using a composite group of tests designed to measure cognitive abilities, consisting of a 5-7 day battery of tests that assessed a wide variety of neuropsychological functions, including memory, language, executive functioning and social cognition. In this study, we focus on the *Armed Forces Qualification Test* (AFQT-7A, DoD 1960). This is a standardized multiple choice test of cognitive aptitude, devised by the Department of Defense. The test measures verbal ability, visual-spatial organization, arithmetic, and functional associations via 100 multiple choice questions. The total score range is from 0-100, and the subtest scores range from 0-25 (Figure 1). It was also the only preinjury cognitive assessment available in this study. The same version of the AFQT was used during preinjury, Phase 2, and Phase 3 assessment.

-----FIGURE 1 ABOUT HERE-----

Initially a correlation analysis was run to assess if current AFQT score was a valid proxy measure for intelligence (as measured by the WAIS-III Full-Scale IQ score taken in P3 of the study). In both head-injured subjects and controls the two were significantly correlated ($r=0.845$, $p<0.001$ head-injured; $r=0.816$, $p<0.001$ controls).

Genetic analysis

See Supplementary Information.

Statistical analysis

A variety of parametric procedures were used in this study. In particular, ANOVAs, linear logistic and stepwise multiple-regression procedures were performed to assess the impact of demographic factors, preinjury intelligence, brain volume loss, lesion location and genetic markers on cognitive ability 36-39 years post-injury, and possible intellectual decline 12-15 years and 36-39 years post-injury. A significance level of $p=0.05$ or less was required to enter and remain in the stepwise regression procedure. This analysis allowed an estimation of the relative contribution of each predictor to each dependent measure's test score or score decline.

RESULTS

ARE NEW AND EXISTING SUBJECTS COMPARABLE?

A t-test was run to compare phase 3 (P3) AFQT scores between P2 and newly recruited controls, and P2 and P1 identified head-injured (HI) subjects. There were no significant differences found. As the newly recruited control subject group consisted of a large number of officers, a similar t-test was run to examine for any differences in intelligence between enlisted and officer controls. There were no differences in terms of any decline, but officers had greater P3 AFQT scores, both in the existing and new control group ($t=-4.000$, $df=233$, $p<0.001$). For statistical purposes, it was decided to include both new and existing control subjects in one group, but to include rank as a fixed factor in any regression analysis. Race was also recoded for caucasians and noncaucasians, because of low numbers in individual racial groups. It should be noted, however, that the newly-recruited control subjects may well represent a different population in terms of other factors that may impact on intelligence, such as social background and tendency to seek support for any cognitive difficulties.

HOW DO HEAD-INJURED AND CONTROL SUBJECTS AFQT SCORES COMPARE?

The median AFQT score at P3 was 65.0. In controls this was 74.0 and in the head-injured 54.0. The median rise in AFQT score from preinjury to P2 was 11.5 in controls and 1.0 in those with head injuries, while from P2 to P3 controls showed a median decline of 4.0 and the head-injured of 7.0.

A t-test was run to compare the change in AFQT scores across 3 time periods (preinjury to P2, P2 to P3, and preinjury to P3). Those with head injuries had a lower current AFQT score than controls ($t=4.265$, $df=246$, $p<0.001$). In the group as a whole, the HI subjects' AFQT score marginally decreased more than controls from P2 to P3 ($t=1.826$, $df=202$, $p=0.069$), and the controls AFQT score increased significantly more from preinjury to P2 ($t=3.047$, $df=194$, $p=0.003$), compared to those with head injuries. However, if the officers were excluded from the sample, the HI subjects' AFQT score significantly decreased more than controls from preinjury to P3 ($t=3.151$, $df=178$, $p=0.002$). The change from P2 to P3 remained marginal in the difference in decline between those with head injuries and controls ($t=1.751$, $df=176$, $p=0.082$) (Figure 2).

-----FIGURE 2 ABOUT HERE-----

WHAT PREDICTS CURRENT (AND CHANGES IN) INTELLIGENCE LEVEL?

A univariate linear regression procedure was performed to assess the predictability of P3 AFQT score, with race, age, military rank, education and VHIS group (i.e. whether a participant was a HI subject or a control) as covariates. Race ($F=24.618$, $df=1$, $p=0.021$),

rank ($F=3.555$, $df=1$, $p=0.031$), VHIS group ($F=7.142$, $df=1$, $p=0.008$), age ($F=4.245$, $df=1$, $p=0.041$) and education ($F=19.667$, $df=1$, $p<0.001$) were all significant in predicting AFQT score. However, when preinjury AFQT score was added as a covariate (or P2 AFQT when looking at P2 to P3 change), only preinjury AFQT score ($F=94.444$, $df=1$, $p<0.001$) and presence of PHI ($F=9.414$, $df=1$, $p=0.003$) were found to have a significant impact on the level of current AFQT. Compared to the previous regression model, VHIS group had greater importance, and race and rank had relatively less impact.

A similar model was used to examine the changes in AFQT score across all time periods (i.e. preinjury to P2, P2 to P3, and preinjury to P3). Looking at change in AFQT score from preinjury to P2, education ($F=4.168$, $df=1$, $p=0.043$ - with a higher level of education predicting a lower degree of drop in AFQT) and preinjury AFQT ($F=18.752$, $df=1$, $p<0.001$) were both significant predictors. VHIS group was marginal in terms of its significance as a predictor ($F=3.338$, $df=1$, $p=0.070$).

Regarding AFQT change from P2 to P3, P2 AFQT ($F=11.453$, $df=1$, $p=0.001$) and VHIS group ($F=7.713$, $df=1$, $p=0.006$) were significant predictors.

When we analyzed AFQT change from preinjury to P3, only preinjury AFQT ($F=27.658$, $df=1$, $p<0.001$) and VHIS group ($F=9.414$, $df=1$, $p=0.003$) were significant as predictors.

When these regression procedures were repeated for HI subjects only, there were no significant changes in the results, except for a slight increased predictability of education when looking at AFQT change from preinjury to P2.

DOES BRAIN VOLUME LOSS (OR ATROPHY) PREDICT INTELLIGENCE LEVEL?

Correlation analyses were used to assess if AFQT score changes were associated with total volume loss (TVL) on CT scan at either P2 (P2 TVL) or P3 (P3 TVL), or the various measures of atrophy (global and regional ratings of atrophy, third ventricle width, change in volume loss from P2 to P3). AFQT score changes from preinjury to both P2 and P3 were significantly correlated with both P2 TVL ($r=-0.367$, $p<0.001$; $r=-0.446$, $p=0.00$) and P3 TVL ($r=-0.330$, $p<0.001$; $r=-0.414$, $p<0.001$). Interestingly, later changes in intelligence (as measured by change in AFQT score from P2 to P3) were not significantly correlated with volume loss at either P2 or P3.

In terms of ratings of atrophy and third ventricle width, third ventricle width was significantly correlated with AFQT score change from preinjury to P3 and preinjury to P2 ($r=-0.236$, $p=0.002$; $r=-0.175$, $p=0.030$), and global atrophy rating **was** significantly correlated with AFQT score change from preinjury to P3 ($r=-0.174$, $p=0.023$). There were also correlations between the current degree of left parietal ($r=-0.222$, $p=0.003$) atrophy and decline in intelligence from preinjury to P3. A logistic regression analysis was carried out to assess if particular areas of atrophy could predict current or change in intelligence. Left parietal ($F=5.178$, $p=0.024$, $df=1$) and right frontal ($F=7.897$, $p=0.006$, $df=1$) atrophy predicted current AFQT score. Change in AFQT score from preinjury to P3 was predicted by degree of left parietal ($F=5.178$, $p=0.024$, $df=1$) and right frontal ($F=7.897$, $p=0.006$, $df=1$) atrophy. From P2 to P3, the only significant predictor of change in intelligence was right parietal atrophy ($F=4.252$, $p=0.041$, $df=1$).

IS THERE EVIDENCE OF DEMENTIA IN THE SAMPLE GROUP?

The Mini-Mental State Examination (MMSE; Folstein *et al.*, 1972) is a commonly used screening tool used to detect significant cognitive decline. A score below 24 out of a

possible 30 is considered indicative of likely dementia (Tariq *et al.*, 2006), yet only 4.5% (n=6) of our subjects had a recorded score of below 24 out of 30 on the MMSE. The median score for controls was 30 and for those with head injuries was 29. Not surprisingly, these subjects with a score of below 24 on the MMSE showed a significantly greater level of decline in intelligence from preinjury to P3 than those with higher scores ($t=-2.458$, $df=116$, $p=0.015$), but they also tended to have a lower preinjury AFQT score ($t=-1.811$, $df=118$, $p=0.073$), suggesting that they may have had some early risk factor for decline. Those with abnormal MMSE scores had significantly larger lesions (mean total volume loss=101.38cc versus 29.64cc for those with higher scores; $t=-4.566$, $df=112$, $p<0.001$), with greater degrees of atrophy ($t=3.292$, $df=109$, $p=0.001$) and wider third ventricles ($t=2.935$, $df=109$, $p=0.004$). A MMSE score below 27 out of 30 is sometimes used to indicate mild cognitive decline (Robert *et al.*, 2006). 15.7% (n=21) of our subjects had a recorded score of below 27 on the MMSE. Those in our sample with a score below 27 had significantly larger lesions than those with the higher scores (mean total volume loss=52.45cc versus 29.10cc for those with higher scores; $t=-2.496$, $df=112$, $p=0.014$). There was no correlation between global rating of atrophy or third ventricle width and MMSE score, although increasing age did correlate with global atrophy ($r=-0.159$, $p=0.030$). There was no significant correlation between age and MMSE score. There was an increased tendency for subjects with low MMSE scores to have the COMT rs2020917 ($\chi^2=6.279$, $p=0.043$) allele, but none of the other genetic markers were related.

We also recorded if subjects had any family history of dementia. This included 16.57% of head-injured subjects and 8.16% of controls. There were no significant differences in terms of current MMSE score, degree of atrophy on CT scan or decline in intelligence from preinjury to P3 in those with a family history of dementia compared to those with no such family history.

Additionally, we noted any current or lifetime prevalence of alcohol abuse or dependence (based on DSM-IV criteria), as it was possible such diagnoses may influence tendency to cognitive decline. In those with head injuries, 30.05% had a lifetime diagnosis of alcohol abuse and 20.20% of dependence. Only 2.59% of those with alcohol diagnoses fulfilled diagnostic criteria at the time of P3. In the controls, 36.54% had a lifetime diagnosis of alcohol abuse and 17.30% of dependence, with 5.77% of those with alcohol diagnoses fulfilled diagnostic criteria at the time of P3. Those with a lifetime history of alcohol use did not have significantly greater levels of decline, lower MMSE scores or greater levels of brain atrophy. Also, when the psychiatric symptoms reported by the next of kin were examined they did not correlate with change in intelligence.

The univariate linear regression procedure containing preinjury intelligence, race, age, rank, education and VHIS group (i.e. whether a participant was a HI subject or a control) as covariates was repeated, adding family history of dementia and lifetime and current alcohol diagnoses into the model. Both history of alcohol diagnoses and family history of dementia had no significant impact on P3 AFQT score.

HOW DO THOSE WITH RIGHT, LEFT AND BILATERAL LESIONS COMPARE?

The number of subjects with left, right and bilateral lesions were similar, as were their demographic data and level of lesion size. There was no significant correlation between lesion laterality and preinjury AFQT and TVL, or current AFQT in all three HI subject groups.

DOES SPECIFIC BRAIN STRUCTURE INVOLVEMENT OR VOLUME LOSS PREDICT AFQT SCORE OR CHANGE IN AFQT?

A stepwise regression analysis was performed to look for predictors of current AFQT and change in AFQT over all phases. The dependant variables included current AFQT scores, right hemisphere volume loss, left hemisphere volume loss, change in total volume loss from P2 to P3, three measurements of corpus collosum width, involvement of the following brain structures: caudate, substantia nigra, globus pallidus, white matter, thalamus, hippocampus, and the specific lateral and overall involvement of the following regions of the cortex: the frontal, parietal, temporal and occipital lobes, as well as the insula and amgdala.

Lesions in the caudate nucleus ($t=-5.623$, $p<0.001$), left parietal lobe ($t=-3.225$, $p=0.002$), right amygdala ($t=2.241$, $p=0.026$), hippocampus ($t=-3.292$, $p=0.001$) and right frontal lobe ($t=-2.055$, $p=0.042$) along with the width of the corpus callosum ($t=-2.992$, $p=0.004$) were predictive of current AFQT score. While change in AFQT score from preinjury to P3 were predicted by lesions in the caudate nucleus ($t=-5.623$, $p=0.006$), left parietal lobe ($t=-3.225$, $p=0.002$), right amygdala ($t=2.241$, $p=0.026$), hippocampus ($t=-3.292$, $p=0.001$) and right frontal lobe ($t=-2.055$, $p=0.042$) along with the width of the corpus callosum ($t=-2.992$, $p=0.004$). All other areas of brain involvement were excluded from further analyses. In terms of change in intelligence from P2 to P3, the only significant predictor was left hemisphere volume loss ($t=-2.188$, $p=0.030$).

DOES PERFORMANCE ON SPECIFIC SUBTESTS OF THE AFQT PREDICT AFQT SCORE OR CHANGE IN AFQT?

A univariate linear logistic regression analyses was completed to assess whether performance in the individual subtests of the AFQT at P3 could predict change in AFQT over time. The AFQT test has four main measures that assess verbal comprehension (vocabulary subtest), visual-spatial imagery (boxes subtest), arithmetic word problems (math subtest), and object-function matching (tools subtest). Performance on three subtests significantly predicted decline in intelligence from P2 to P3: boxes ($F=20.371$, $df=1$, $p<0.001$), math ($F=18.816$, $df=1$, $p<0.001$) and tools ($F=10.675$, $df=1$, $p=0.001$). From preinjury to P3, performance on all four subtests predicted decline in intelligence (vocabulary: $F=10.073$, $df=1$, $p=0.002$; boxes: $F=29.085$, $df=1$, $p<0.001$; math: $F=28.870$, $df=1$, $p<0.001$; tools: $F=19.376$, $df=1$, $p<0.001$).

A stepwise regression analysis was performed to examine whether performance on individual subtests of the AFQT interacted with lesion location to predict decline in AFQT scores from P2 to P3. The boxes subtest, math subtest and tools subtest **only** interacted with left parietal lobe lesions ($R^2=0.421$, $t=-2.056$, $p=0.041$) to predict P2 to P3 decline in the overall AFQT score. Similarly, from preinjury to P3, all the AFQT subtests interacted significantly with lesions in the left parietal lobe to predict AFQT score decline ($R^2=0.645$, $t=-2.550$, $p=0.012$).

DO GENETIC MARKERS PREDICT AFQT SCORE CHANGE?

We found broadly similar incidences of the genetic polymorphisms in our sample compared to other human studies (See Supplemental Information). Crawford et al (2002) found 72.7% lacked APO E4 in a head-injured sample; a result not significantly different from our group. For COMT, other studies have found an incidence of Val/Val at 27-40%, 42-55% for Val/Met and 18-21% for Met/Met in the target population, and our numbers

were also not deviant from that expected for genotypes in the Hardy-Weinberg equilibrium (Egan *et al.*, 2001; Malhotra *et al.*, 2002; Stefanis *et al.*, 2005). Additionally, regarding BDNF, Chuu *et al.* (2006) and Laske *et al.* (2006) reported similar rates of genotypes to this sample. The remaining genetic polymorphisms have had limited testing in human head-injured populations. A linear logistic regression analyses was completed to assess the predictability of preinjury AFQT, P3 AFQT and change in AFQT from preinjury to both P2 and P3 based on a number of genetic markers that have been associated with response to brain injury (APO E4, COMT, GRIN, BDNF, GAD and DBH), together with race, age, rank, education and VHIS group (i.e. whether a participant was a HI subject or a control) as covariates. These analyses were repeated in the head-injured subjects alone, as well in the entire sample.

Preinjury AFQT Score

A large number of the genetic markers we examined predicted preinjury AFQT score. These included GRIN2C rs689730 ($F=3.615$; $p=0.029$, $df=2$), GAD2 rs2839670 ($F=6.815$; $p=0.010$, $df=1$) and DBH444 ($F=3.239$ $p=0.042$, $df=2$). GRIN2B rs1805482 ($F=2.972$; $p=0.054$, $df=2$), COMT: rs9332330 ($F=2.597$, $p=0.078$, $df=2$) and the presence of an APOE 4 allele ($F=3.238$, $p=0.074$, $df=1$) came close to significance in their ability to predict preinjury intelligence score. The total amount of variance in preinjury AFQT score accounted for by these genetic markers was assessed by repeating the logistic regression analyses, including just those genetic markers highlighted above with and without the variable of racial grouping only (as it was anticipated that the other covariates, such as years of education, would have a comparatively later impact on performance). When racial group was assessed alone it produced a R^2 value of 0.180, and when the genetic markers were added this value increased to 0.335, implying that the presence of all of these markers could account for a further 15.5% (33.5% minus 18.0%)

of the variability in preinjury intelligence. It should be noted however, that this analysis involved the relevant genetic markers being entered into the model in a stepwise arrangement, with the R^2 value reflecting the order of presentation of each marker as described above.

Current AFQT Score

Only GRIN2A rs968301 was found to be able to predict current AFQT score at a significant level ($F=3.802$; $p=0.025$, $df=2$).

Change in AFQT score from preinjury to P2

Two of the GAD markers significantly predicted AFQT score change; GAD1 rs11682957 ($F=4.673$; $p=0.011$, $df=2$) and GAD1 rs2241165 ($F=3.182$; $p=0.045$, $df=2$). COMT rs9332330 also significantly predicted recovery of AFQT score ($F=4.259$; $p=0.016$, $df=2$), with the analysis suggesting homozygotes had a better recovery of function based on AFQT score compared with heterozygotes (homozygotes, $B = -7.234$).

Change in AFQT score from P2 to P3

The only genetic marker that was found to significantly predict overall change in AFQT score from P2 to P3 was GRIN2A rs968301 ($F=4.033$; $p=0.020$, $df=2$). All subjects with GRIN2A rs968301 were either homozygous dominant (A1), homozygous recessive (A2) or were heterozygotes. An ANOVA was carried out to see if there was a significant difference in decline based on genotype. Whilst there was a trend for dominant homozygotes to have a greater level of decline in intelligence from P2 to P3 (mean=-

11.714, sd=15.663) compared with recessive homozygotes (mean=-9.071, sd=12.608) and heterozygotes (mean=-8.519, sd=10.988), this did not reach significance.

Change in AFQT score from preinjury to P3

Similarly, the only genetic marker that was found to significantly predict overall change in AFQT score from preinjury to P3 was GRIN2A rs968301 ($F=3.802$; $p=0.025$, $df=2$). An ANOVA procedure was carried out to see if those with a GRIN2A rs968301 allele showed an increased level of decline in intelligence from preinjury to P3. Again although there was a trend for dominant homozygotes to have a greater level of decline in intelligence from P2 to P3 (mean=-9.429, sd=22.849) compared with recessive homozygotes (mean=-8.021, sd=18.432) and heterozygotes (mean=-5.548, sd=18.205), this did not reach significance.

We also repeated the above analyses on subjects in the caucasian racial group, to ascertain whether any of the genetic variability found was robust enough to be present even when racial background was removed from the model. The results were similar to above with the identical genetic markers as described above found to have a significant impact on both current and change in intelligence across the various stages. The only exception was that GAD1 rs11682957 was no longer found to significantly predict change in AFQT score from preinjury to P2 ($F=2.611$; $p=0.078$, $df=2$).

WHAT ARE THE FACTORS THAT BEST PREDICT EXACERBATED DECLINE IN P3 COMPARED TO PREINJURY AND P2 AFQT SCORES?

We computed a linear logistic regression analysis including all the factors found to be predictive of alterations in AFQT score in the previous analyses reported in this paper.

These included; preinjury AFQT, total years of education, right amygdala involvement, caudate involvement, left parietal involvement, right frontal involvement, hippocampal involvement, left temporal involvement, globus pallidus involvement, third ventricle width, global rating of atrophy, width of the corpus collosum (all based on measurements taken at P3) and GRIN2A rs968301.

When looking at the overall changes in intelligence from preinjury to P3, the following were significant in their prediction of decline (in order according to regression coefficient value); right amygdala involvement ($B=-28.261$, $F=10.546$, $df=1$, $p=0.001$), hippocampal involvement ($B=16.851$, $F=9.840$, $df=1$, $p=0.002$), caudate involvement ($B=9.558$, $F=5.798$, $df=1$, $p=0.017$), left parietal involvement ($B=8.984$, $F=7.066$, $df=1$, $p=0.009$) and preinjury AFQT ($B=-0.293$, $F=27.523$, $df=1$, $p<0.001$). For change in AFQT score from P2 to P3, the only factor that was significant in its predictability when the same factors were entered was AFQT score at P2 ($B=-0.150$, $F=8.932$, $df=1$, $p=0.003$). Given that GRIN2A genotype variation has been found to potentially influence the age of onset in Huntington's Disease (Arning et al 2005), we hypothesized that the caudate nucleus linked to GRIN2A genotype. Thus we repeated the analysis without the inclusion of caudate involvement as a covariate, but the results did not differ.

Finally, we carried out a stepwise linear regression analysis to identify the relative contributions each significant factor may have had in predicting change in intelligence (Tables 2-4). In terms of AFQT at P3, the predictors in order of significance were preinjury AFQT (which was found to account for 51% of the proportion of variance in P3 AFQT test scores), caudate involvement (8.2%), left parietal involvement (2.3%), hippocampal involvement (2.3%), corpus collosum distance (2%), right amygdala

involvement (1.1%) and right frontal involvement (1%). In combination, these factors accounted for nearly 70% of the variation in P3 AFQT test scores. For change from P2 to P3, the only significant predictor was AFQT score at P2. For change in intelligence from preinjury to P3, the predictors accounted for less of the variance. They were, in order of significance, were preinjury AFQT (12.7%), caudate involvement (14.7%), left parietal involvement (4.2%), corpus collosum distance (3.6%), hippocampal involvement (4%), right amygdala involvement (2%) and right frontal involvement (1.8%).

-----TABLES 2,3,4 ABOUT HERE-----

DISCUSSION

We found evidence that patients with PHI, compared to matched controls, demonstrate exacerbated decline in general intelligence. This is consistent with a number of previous studies that supported the concept of a process of exacerbated decline after TBI (Corkin *et al.*, 1989; Klein *et al.*, 1996; Himanen, 2006). Yet, among our head-injured participants we found those with a lower preinjury and P2 AFQT, as well as lower levels of education tended not to attend P3 of the study. This implies a selection bias, and thus we have only been able to review the long term demographic, clinical and genetic predictors in those Vietnam Veterans who were probably least at risk from the outset (Grafman *et al.*, 1988). If the theory of cognitive reserve is valid, we may well have seen differing results if we had been able to examine the entire head-injured sample in P3 of the study. We did not, however, find evidence of increasing levels of frank dementia, maybe as a result of the still relatively young age of the majority of the participants.

Our data show that AFQT scores at the time of military induction, along with total years of education, were the two greatest predictors of P3 AFQT scores for both PHI and

control groups. A higher AFQT score before injury and increased educational attainment acted in a protective manner against decline in general intelligence post-PHI. Age was a less significant predictor, but also had an impact, with increasing age leading to a greater decline in intelligence. Change in AFQT score in the first two decades after injury was most associated with preinjury intelligence, and to a lesser extent educational duration. Educational level appears to have an impact earlier in the process of recovery from head injury. However, AFQT score prior to injury remained the greatest forecaster of overall cognitive outcome almost four decades after a PHI. This mirrors the findings in P2 of the VHIS (Grafman *et al.*, 1988), and studies that have linked early TBI with accelerated cognitive decline later in life (Klein *et al.*, 1996). Given that we have replicated other research that is not specific to PHI, we would cautiously suggest that the results in our sample of veterans with PHI may be generalized to those with both closed and penetrating TBI.

We found no evidence that laterality of lesion affected level of overall current intelligence or decline. Nor did specific brain structure lesions predict change in AFQT score from P2 to P3. However, with respect to change in AFQT score from preinjury to P3, we found an association between corpus callosum thickness, third ventricle width and rating of global atrophy and both current AFQT and change in AFQT score. Involvement of the caudate nucleus, left parietal lobe, right amygdala, hippocampus, and right frontal lobe in a PHI appeared to influence level of performance over the 30 years post injury. In addition, we found that lesions in the left parietal region interacted with performance on the math, boxes and tools subtests of the AFQT. The brain regions we found to have a significant role in terms of decline in intelligence can be related to the subtests of the AFQT test, performance on all of which was correlated with change in intelligence over time. The caudate nucleus has been found to have a role in language comprehension (Grossman *et al.*, 2002). The parietal lobes are associated with visuospatial judgements

(Ekstrom *et al.*, 2003; Sack *et al.*, 2007), sometimes in conjunction with networks involving the frontal lobes (Chun and Turk-Browne, 2007). The hippocampus is of course vital for memory and attentional tasks (Aalto *et al.*, 2005), and has been linked to networks involving the amygdala in processing of emotional information (Richardson *et al.*, 2003).

Interestingly, we also found an association between atrophy in the left parietal and right frontal regions and degree of decline in intelligence. It should be noted that the atrophy most often occurred adjacent to the location of the original lesion and in the corresponding lobe. Given the marginal group differences in decline between P2 and P3, what was apparent in the head injured group was a slow decline that started post injury against a foreground of recovery of function. So it is feasible that what we are calling an exacerbated decline likely had its origins in the injury and not a dementia, and could have been modified by severity (i.e. volume loss) and location of injury, education, intellectual development, and genetic endowment.

In terms of genetic predictors, we found no apparent associations between APO e4 and cognitive degeneration, as in some other studies, suggesting that exacerbated decline is a phenomena independent of at least some forms of Alzheimer's disease. Our analysis of the data involving the COMT genotypes was similar to other studies, in that we found that having the Val/Val polymorphism led to the most pronounced intellectual decline. However, prior studies have reported associations with the known functional variant COMT Val158Met (rs4680). It should be noted that in the current study, an association was seen between low MMSE score and the intronic marker rs2020917 which is in a different LD block than rs4680. Of all the polymorphisms examined, we found that GRIN2A rs968301, was the most important predictor of exacerbated decline. Interestingly, GRIN2A genotype variation has been found to potentially influence the age

of onset in Huntington's Disease (Arning et al 2005). GRIN 2A is one of the genes that codes for the different subunits of NMDA receptors, including NR2A. NMDA receptor subunit composition has also been found to predict brain plasticity, with NR2A being linked to reduced plasticity (Barria and Malinow, 2005). Additionally, we showed a link between GRIN, GAD, DBH and COMT and preinjury intelligence, suggesting that these genotypes may have their greatest influence via their effects on protective mechanisms in PHI. By performing our analyses in the entire population, it is possible that the findings could have been affected by potential population stratification. However, we found similar results regarding the impact of genetic markers when the analysis was rerun on the caucasian subpopulation, so that would have would reduced the false positive due to allele frequency differences between populations. These findings show that genetic markers may play a small but significant role in different stages of cognitive recovery or decline after head injury.

We have presented a number of important findings from this large, long term follow up study of subjects with penetrating brain injuries. Our findings suggest that exacerbated decline in intelligence is a significant risk for those with PHI, but that intelligence prior to PHI is the most vital predictor of outcome thirty years after the injury. However, we have also been able to demonstrate that specific regions of brain damage affect this change, as does the degree of local and global atrophy, even in the absence of frank dementia. Additionally, this is the first study to examine genetic factors in the long term outcome following PHI. Our findings indicate that genotype variations do play a role in exacerbated decline after head injury. Clinicians treating veterans with PHI should evaluate any changes in their neurobehavioral status carefully so as to not confuse an exacerbated decline in function with frank dementia. This additional burden to brain-injured veterans should be considered when estimating their future health care needs.

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LEGEND

Supplemental Information

Table 1 – Comparison of head-injured and control subjects at P3

Table 2 – Relative contribution to change in intelligence made by significant predictive factors

Table 3 – Relative contribution to change in AFQT from P2 to P3 made by significant predictive factors

Table 4 – Relative contribution to change in AFQT from preinjury to P3 made by significant predictive factors

Figure 1 – Sample of questions from the AFQT test (A=vocabulary subtest; B=arithmetic subtest; C=tools subset; D=boxes subtest)

Figure 2 - Mean change in AFQT score from P2 to P3 according to subject grouping

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SUPPLEMENTAL INFORMATION

Genomic DNA. Genomic DNA was isolated from blood leukocytes using a Nucleon™ BACC2 kit according to the manufacture's protocol (Amersham Life Science, Piscataway, NJ). Quality and quantity of genomic DNA was determined spectrophotometrically using the absorbance reading 260 nm and 280 nm. Some DNA samples were re-purified by incorporating an additional phenol:chlorform (24:1 v/v) extraction prior to recovery by ethanol precipitation. DNA concentrations were measured using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). The completion rate of each assay was > 99%, with an error rate of < 1%.

ApoE genotyping. Genotyping of apoE alleles used a pre-developed ABI assay (Assay-on-Demand) 5'nuclease assay to distinguish between each of the polymorphisms occurring at codons 130 and 176 of the coding sequence from the apoE gene (NCBI nucleotide accession number M10065). The assay distinguishes between each of the know e2, e3, and e4 alleles where the combinations 130Cys/176Cys, 130Cys/176Arg, and 130Arg/176Arg give rise to the E2, E3, and E4 alleles, respectively. The detection oligonucleotide sequences were: codon 130 (dbSNP ID rs429358) 5'-GCTGGGCGCGGACATGGAGGACGTG[C/T]GCGGCCGCCTGGTGCAGTACCGC GG-3'. The C-Allele incorporated FAM while the T-Allele used VIC; codon 176 (dbSNP ID rs7412) 5'-CCGCGATGCCGATGACCTGCAGAAG[C/T]GCC TGGCAGTGTACCAGGCCGGGGC-3'. The C-Allele was labeled with FAM while the T-Allele was labeled with VIC. The following positions on the human COMT gene were analyzed: ApoE: rs429358, ApoE: rs7412, ApoE: rs769452. Additionally the number of ApoE haplotypes and the presence or absence of ApoE E2, E3 and E4 were assessed.

COMT genotyping. A 5' nuclease assay using fluorogenic detection probes was performed based on the G1947A single nucleotide polymorphism within exon 4 of the human COMT gene (NCBI nucleotide accession number Z26491), corresponding to codon 158 of the COMT gene (NCBI accession number BC011935). The detection oligonucleotide sequences were: 5'-Fam6-CCTTGTCTTCATGCCAGCGA-TAMRA-3' (Val158 detection probe) and 5'-Vic-ACCTTGTCTTCATGCCAGCGAA AT-TAMRA-3' (Met158 detection probe). FAM is 6-carboxyfluorescein and TAMRA is 6-carboxytetramethylrhodamine. The variant nucleotide in each detection probe is shown in lower case. The oligonucleotide primers used for amplification were 5'-TCGAGATCAACCCC GACTGT-3' (forward) and 5'-AACGGGTCAGGCATGCA-3' (reverse). Target DNA amplification, fluorescence measurements, and allele discrimination were accomplished using a ABI 7900 Sequence Detection System (Applied Biosystems, Foster City, CA). The following positions on the human COMT gene were analyzed: COMT: rs4680, COMT: rs9332330, COMT: rs2020917.

GRIN genotyping. The following positions on the human GRIN gene were analyzed: GRIN1: rs2301364, GRIN1: rs4880213, GRIN1: rs1126448, GRIN1: rs3181457, GRIN1: rs11575901, GRIN2A: rs1014531, GRIN2A: rs8050843, GRIN2A: rs1420040, GRIN2A: rs968301, GRIN2A: rs11074504, GRIN2A: rs2302711, GRIN2A: rs1071504, GRIN2B: rs3764030, GRIN2B: rs1805482, GRIN2B: rs1806201, GRIN2C: rs3744215, GRIN2C: rs7219247, GRIN2C: rs2683267, GRIN2C: rs689730.

GAD genotyping. The following positions on the human GAD gene were analyzed: GAD1: rs11682957, GAD1: rs2241165, GAD1: rs3749034, GAD1: rs12185692, GAD1: rs769395, GAD2: rs876848, GAD2: rs2839670, GAD2: rs1330582, GAD2: rs1330579, GAD2: rs3781106.

BDNF genotyping. The following positions on the human BDNF gene were analyzed: BDNF-LNG, BDNF: 1197557, BDNF: 11592757, BDNF: 11592758. *BDNF* val₆₆met genotypes were determined using a 5' – exonuclease allelic discrimination (Taqman) assay using Reference SNP ID: rs6265 (ABI Assay on Demand C_11592758_10, Applied Biosystems, Foster City, CA), on an ABI7900 instrument. Genotyping error rate for this assay was determined by replicate genotyping of samples, and was < 0.005.

DBH genotyping. The following position on the human DBH gene was analyzed: DBH444.

INCIDENCE OF SPECIFIC GENETIC POLYMORPHISMS IN SAMPLE

POPULATION

Genetic polymorphism	A1 present (N/%)	Both (N/%)	A2 present (N/%)
GRIN2A rs1014531	76/29.9	106/41.7	33/13.0
GRIN2A rs8050843	104/40.9	85/33.5	25/9.8
GRIN2A rs1420040	39/15.4	107/42.1	68/26.8
GRIN2A rs968301	21/8.3	97/38.2	96/37.8
GRIN2A rs11074504	74/29.1	83/32.7	49/19.3
GRIN2A rs2302711	89/35.0	87/34.3	25/9.8
GRIN2A rs1071504	91/35.8	117/46.1	10/3.9
GRIN2B rs3764030	10/3.9	63/24.8	135/53.1
GRIN2B rs1805482	120/47.2	74/29.1	18/7.1
GRIN2B rs1806201	113/44.5	83/32.7	19/7.5
GRIN2C rs3744215	24/9.4	78/30.7	114/44.9
GRIN2C rs7219247	23/9.1	79/31.1	114/44.9
GRIN2C rs2683267	114/44.9	79/31.1	22/8.7
GRIN2C rs689730	176/69.3	35/13.8	3/1.2
COMT rs4680	Val/Val=48/18.9	Val/Met=99/39.0	Met/Met=69/27.2
COMT rs9332330	19/7.5	79/31.1	101/39.8
GAD1 rs3749034	129/50.8	73/28.7	6/2.4
GAD1 rs12185692	75/29.5	108/42.5	32/12.6
GAD1 rs769395	13/5.1	88/34.6	115/45.3
GAD2 rs876848	137/53.9	65/25.6	14/5.5
GAD2 rs2839670	20/7.9	192/75.6	212/83.5
GAD2 rs1330582	11/4.3	61/24.0	138/54.3

GAD2 rs1330579	215/84.6	N/A	N/A
	No	Yes	
ApoE E2 allele present	181/71.3	36/14.2	
ApoE E3 allele present	14/5.5	203/79.9	
ApoE E4 allele present	166/65.4	51/20.1	
	1,1	1,2	2,2
SOD2: Diplotypes	38/15.0	32/12.6	9/3.5
(1,1)(1,2)(2,2)			
	1,1	1,3	3,3
SOD2: Diplotypes	38/15.0	29/11.4	9/3.5
(1,1)(1,3)(3,3)			
	0	1	2
SOD2: Copies of Haplotype 1	38/15.0	61/24.0	38/15.0
SOD2: Copies of Haplotype 2	76/29.9	52/20.5	9/3.5
SOD2: Copies of Haplotype 3	79/31.1	49/19.3	9/3.5

**GENETIC MARKERS CLASSIFIED ACCORDING TO DBSNP IDENTIFIERS
(RS #) AND CELERA CODE**

Gene	dbSNP ID	Celera ID	Allele	Design		SNP Type
				strand	Chr	
APOE	rs1799981		[C/T]	Forward	19	PROMOTER
APOE	rs429358	hCV3084793	[C/T]	Reverse	19	MIS-SENSE MUTATION
APOE	rs7412	hCV904973	[C/T]	Reverse	19	MIS-SENSE MUTATION
APOE	rs769452	hCV904985	[C/T]	Reverse	19	MIS-SENSE MUTATION
BDNF	LNG-SNP		[C>T]	Forward	11	MIS-SENSE MUTATION
BDNF	rs1491851	hCV1197557	[C/T]	Reverse	11	INTERGENIC/UNKNOWN
BDNF	rs1519480	hCV11592757	[C/T]	Forward	11	INTERGENIC/UNKNOWN
BDNF	rs6265	hCV11592758	[C/T]	Reverse	11	MIS-SENSE MUTATION
COMT	rs9332330	hCV2255423	[C/T]	Forward	22	INTRON
COMT	rs2020917	hCV11731880	[T/C]	Reverse	22	INTRON
COMT	rs4680	hCV25746809	[A/G]	Reverse	22	MIS-SENSE MUTATION
GAD1	rs11682957	hCV2177420	[C/G]	Reverse	2	INTERGENIC/UNKNOWN
GAD1	rs12185692	hCV2177461	[A/C]	Forward	2	INTERGENIC/UNKNOWN
GAD1	rs2241165	hCV2177447	[C/T]	Forward	2	INTRON
GAD1	rs3749034	hCV2177452	[A/G]	Reverse	2	UTR 5
GAD1	rs769395	hCV8823522	[A/G]	Reverse	2	UTR 3
GAD2	rs1330579	hCV8868495	[C/G]	Forward	10	INTRON
GAD2	rs1330582	hCV1443809	[C/T]	Reverse	10	INTRON
GAD2	rs2839670	hCV1443775	[C/A]	Forward	10	INTERGENIC/UNKNOWN
GAD2	rs3781106	hCV11562835	[G/A]	Reverse	10	INTRON
GAD2	rs876848	hCV1443740	[A/G]	Forward	10	INTERGENIC/UNKNOWN
GRIN1	rs1126448		[G>T]	Forward	9	MIS-SENSE MUTATION

GRIN1	rs11575901		[C>T]	Forward	9	MIS-SENSE MUTATION
GRIN1	rs2301364	hCV15756180	[C/T]	Forward	9	INTRON
GRIN1	rs3181457		[T>G]	Forward	9	MIS-SENSE MUTATION
GRIN1	rs4880213	hCV27929891	[C/T]	Reverse	9	INTERGENIC/UNKNOWN
GRIN2A	rs1014531	hCV26609067	[A/G]	Forward	16	UTR 3
GRIN2A	rs1071504	hCV2993214	[C/T]	Reverse	16	INTERGENIC/UNKNOWN
GRIN2A	rs11074504	hCV26609051	[G/T]	Forward	16	INTRON
GRIN2A	rs1420040	hCV2973912	[A/G]	Reverse	16	INTERGENIC/UNKNOWN
GRIN2A	rs2302711	hCV2925633	[C/T]	Forward	16	INTRON
GRIN2A	rs8050843	hCV29181223	[C/T]	Reverse	16	INTRON
GRIN2A	rs968301	hCV8928212	[C/T]	Reverse	16	INTRON
GRIN2B	rs1805482	hCV16204621	[A/G]	Forward	12	SILENT MUTATION
GRIN2B	rs1806201	hCV25472760	[A/G]	Reverse	12	MIS-SENSE MUTATION
GRIN2B	rs3764030	hCV27497950	[C/T]	Forward	12	INTERGENIC/UNKNOWN
GRIN2C	rs2683267	hCV1197172	[C/T]	Forward	17	INTRON
GRIN2C	rs3744215	hCV25804881	[C/A]	Reverse	17	MIS-SENSE MUTATION
GRIN2C	rs689730	hCV1197175	[A/G]	Forward	17	SILENT MUTATION
GRIN2C	rs7219247	hCV29395817	[C/T]	Forward	17	INTRON
HTT	rs1042173		[G>T]	Forward	17	3' UTR
HTT	SL+rs25531		S/LA/LG	Forward	17	PROMOTER

**TABLE 1 – COMPARISON OF HEAD-INJURED AND CONTROL SUBJECTS
AT P3**

		Mean	Std. Deviation	p
Age at testing	Control	59.15	3.873	0.061
	Head-injured	58.11	2.940	
	Total	58.31	3.155	
Years of education	Control	14.16	2.398	0.922
	Head-injured	14.20	2.270	
	Total	14.19	2.283	
Preinjury intelligence	Control	65.40	22.91	0.238
	Head-injured	59.91	25.54	
	Total	6078	25.18	

**TABLE 2 – RELATIVE CONTRIBUTION TO AFQT AT P3 MADE BY
SIGNIFICANT PREDICTIVE FACTORS**

Model		B	Std. Error	t	p	R squared	R squared change
1	(Constant)	7.808	3.746	2.084	.039		
	Preinjury AFQT	.728	.056	12.936	.000	.510	.510
2	(Constant)	11.386	3.485	3.267	.001		
	Preinjury AFQT	.730	.051	14.170	.000		
	Phase 3 CT: Caudate involvement	-18.193	3.201	-5.684	.000	.592	.082
3	(Constant)	12.387	3.409	3.634	.000		
	Preinjury AFQT	.747	.050	14.805	.000		
	Phase 3 CT: Caudate involvement	-16.462	3.167	-5.199	.000		
	Left Parietal Involvement	-8.979	2.884	-3.114	.002	.615	.023
4	(Constant)	21.745	4.593	4.734	.000		
	Preinjury AFQT	.744	.049	15.109	.000		
	Phase 3 CT: Caudate involvement	-15.522	3.108	-4.993	.000		
	Left Parietal Involvement	-8.728	2.817	-3.098	.002		
	Phase 3 CT: Distance 2 of CC	-1.497	.506	-2.957	.004	.636	.020
5	(Constant)	21.962	4.536	4.842	.000		
	Preinjury AFQT	.742	.049	15.248	.000		
	Phase 3 CT: Caudate involvement	-17.819	3.234	-5.510	.000		
	Left Parietal	-8.039	2.798	-2.873	.005		

	Involvement						
	Phase 3 CT: Distance						
	2 of CC	-1.552	.500	-3.101	.002		
	Phase 3 CT: Right					.647	.011
	Amygdala	15.305	6.787	2.255	.026		
	involvement						
6	(Constant)	22.251	4.403	5.053	.000		
	Preinjury AFQT	.746	.047	15.789	.000		
	Phase 3 CT: Caudate						
	involvement	-12.402	3.551	-3.493	.001		
	Left Parietal						
	Involvement	-6.921	2.738	-2.528	.012		
	Phase 3 CT: Distance						
	2 of CC	-1.615	.486	-3.323	.001		
	Phase 3 CT: Right						
	Amygdala	26.335	7.404	3.557	.000		
	involvement						
	Phase 3 CT:					.670	.023
	Hippocampus	-15.812	4.846	-3.263	.001		
	involvement						
7	(Constant)	24.466	4.466	5.479	.000		
	Preinjury AFQT	.740	.047	15.814	.000		
	Phase 3 CT: Caudate						
	involvement	-11.899	3.515	-3.385	.001		
	Left Parietal					.680	.010
	Involvement	-7.983	2.747	-2.906	.004		
	Phase 3 CT: Distance						
	2 of CC	-1.419	.488	-2.904	.004		
	Phase 3 CT: Right						
	Amygdala	28.642	7.390	3.876	.000		
	involvement						

Phase 3 CT:						
Hippocampus involvement	-17.123	4.824	-3.549	.001		
Right Frontal Involvement	-5.375	2.446	-2.198	.029		

TABLE 3 – RELATIVE CONTRIBUTION TO CHANGE IN AFQT FROM P2 TO P3 MADE BY SIGNIFICANT PREDICTIVE FACTORS

Model	B	Std. Error	t	p	R squared	R squared change
1 (Constant)	-1.682	2.527	-.666	.507		
P2 AFQT	-.120	.037	-3.248	.001	.064	.064

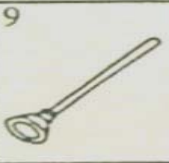
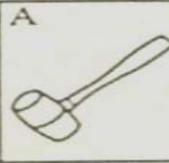
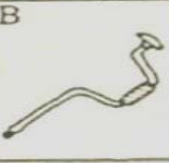
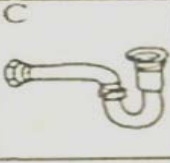
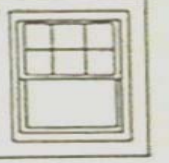
TABLE 4 – RELATIVE CONTRIBUTION TO CHANGE IN AFQT FROM PREINJURY TO P3 MADE BY SIGNIFICANT PREDICTIVE FACTORS

Model		B	Std. Error	t	p	R squared	R squared change
1	(Constant)	7.808	3.746	2.084	.039		
	Preinjury AFQT	-.272	.056	-4.837	.000	.127	.127
2	(Constant)	11.386	3.485	3.267	.001		
	Preinjury AFQT	-.270	.051	-5.253	.000		
	Phase 3 CT: Caudate involvement	-18.193	3.201	-5.684	.000	.274	.147
3	(Constant)	12.387	3.409	3.634	.000		
	Preinjury AFQT	-.253	.050	-5.017	.000		
	Phase 3 CT: Caudate involvement	-16.462	3.167	-5.199	.000		
	Left Parietal Involvement	-8.979	2.884	-3.114	.002	.315	.042
4	(Constant)	21.745	4.593	4.734	.000		
	Preinjury AFQT	-.256	.049	-5.187	.000		
	Phase 3 CT: Caudate involvement	-15.522	3.108	-4.993	.000		
	Left Parietal Involvement	-8.728	2.817	-3.098	.002		
	Phase 3 CT: Distance 2 of CC	-1.497	.506	-2.957	.004	.351	.036
5	(Constant)	21.962	4.536	4.842	.000		
	Preinjury AFQT	-.258	.049	-5.304	.000		
	Phase 3 CT: Caudate involvement	-17.819	3.234	-5.510	.000		
	Left Parietal Involvement	-8.039	2.798	-2.873	.005		
	Phase 3 CT: Distance 2 of CC	-1.552	.500	-3.101	.002		

6	Phase 3 CT: Right Amygdala involvement	15.305	6.787	2.255	.026	.372	.020
	(Constant)	22.251	4.403	5.053	.000		
	Preinjury AFQT	-.254	.047	-5.377	.000		
	Phase 3 CT: Caudate involvement	-12.402	3.551	-3.493	.001		
	Left Parietal Involvement	-6.921	2.738	-2.528	.012		
	Phase 3 CT: Distance 2 of CC	-1.615	.486	-3.323	.001		
	Phase 3 CT: Right Amygdala involvement	26.335	7.404	3.557	.000		
7	Phase 3 CT: Hippocampus involvement	-15.812	4.846	-3.263	.001	.412	.040
	(Constant)	24.466	4.466	5.479	.000		
	Preinjury AFQT	-.260	.047	-5.569	.000		
	Phase 3 CT: Caudate involvement	-11.899	3.515	-3.385	.001		
	Left Parietal Involvement	-7.983	2.747	-2.906	.004		
	Phase 3 CT: Distance 2 of CC	-1.419	.488	-2.904	.004		
	Phase 3 CT: Right Amygdala involvement	28.642	7.390	3.876	.000		
	Phase 3 CT: Hippocampus involvement	-17.123	4.824	-3.549	.001		
	Right Frontal Involvement	-5.375	2.446	-2.198	.029	.429	.018

FIGURE 1 – SAMPLE OF QUESTIONS AFQT

<p>A</p> <p>1. The boys <u>discovered</u> the cave.</p> <p>(A) Searched (B) Found (C) Enlarged (D) Entered</p>	<p>B</p> <p>5. Bob wants to buy a wagon. He has 5 dollars and needs 5 dollars more. How much does the wagon cost?</p> <p>(A) \$10 (B) \$15 (C) \$25 (D) \$55</p>
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9	A	B	C	D
				

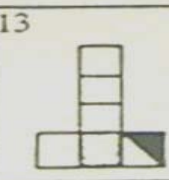
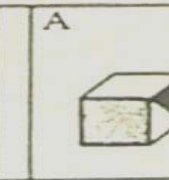
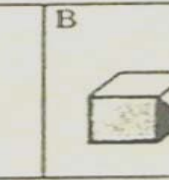
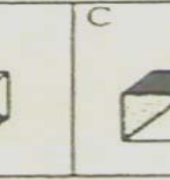
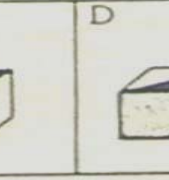
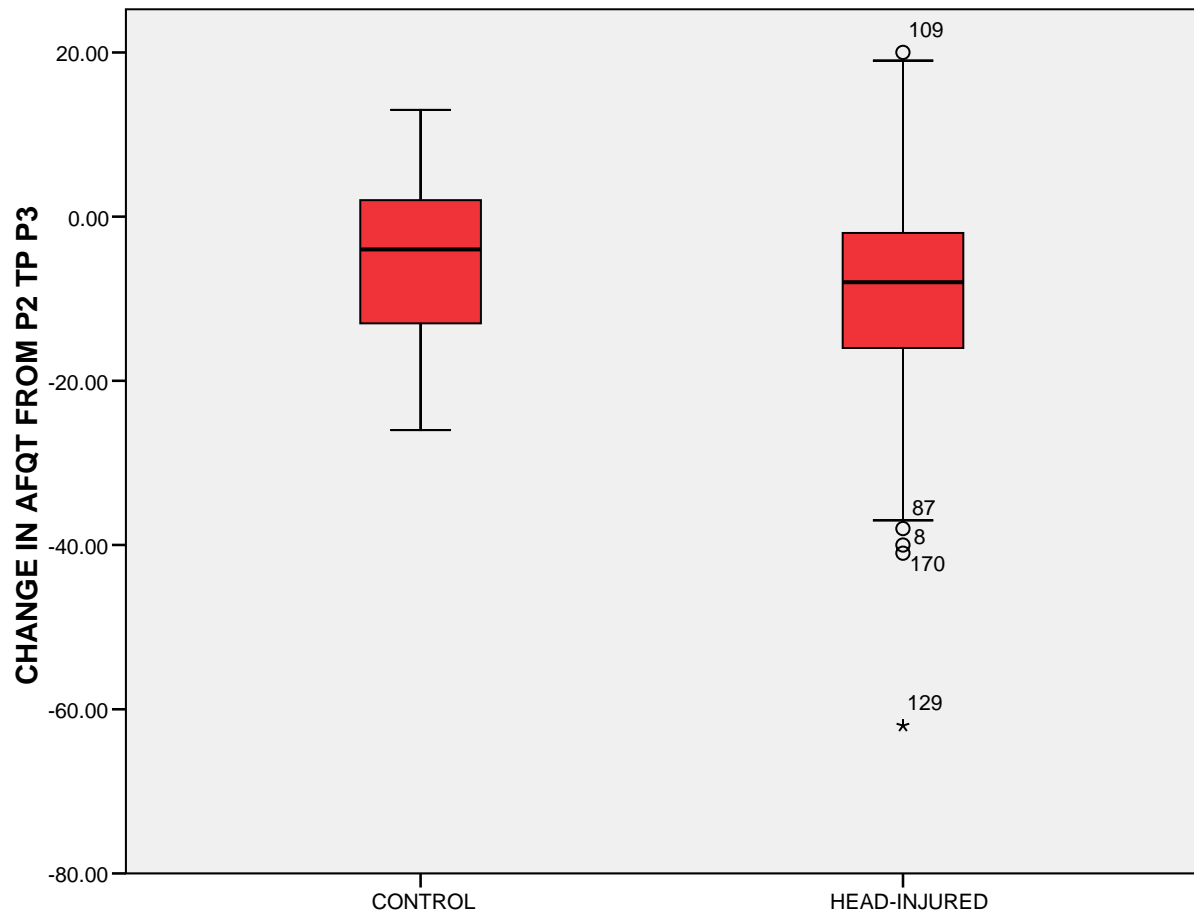
13	A	B	C	D
				

FIGURE 2 - MEAN CHANGE IN AFQT SCORE FROM P2 TO P3 ACCORDING TO SUBJECT GROUPING



APPENDIX B

Focal brain damage protects against post-traumatic stress disorder in combat veterans

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Post-traumatic Stress Disorder (PTSD) is an often debilitating mental illness characterized by recurrent distressing memories of traumatic events¹. PTSD is associated with hypoactivity in ventromedial prefrontal cortex (vmPFC)²⁻⁷, hyperactivity in amygdala^{4-6, 8-10}, and reduced volume in hippocampus^{5,6}, but it is unknown whether these neuroimaging findings reflect the underlying cause of the disorder, or a secondary effect. To investigate the causal contribution of specific brain areas to PTSD symptomology, we studied a unique sample of Vietnam War veterans who suffered brain injury and emotionally traumatic events. We found a significantly reduced occurrence of PTSD following vmPFC damage and the complete absence of PTSD following amygdala damage. These results show that vmPFC and amygdala are critically involved in the pathogenesis of PTSD, and suggest that interventions aimed at selectively disrupting vmPFC and/or amygdala function could have efficacy to treat PTSD.

Post-traumatic Stress Disorder (PTSD) is characterized by re-experience of a traumatic event (e.g., flashbacks), emotional numbing, avoidance of reminders of the event, and hyperarousal (e.g., excessive vigilance)¹. With an estimated prevalence of

over 15 million, PTSD is a major global health problem, and is among the ten medical conditions most likely to cause sufferers to miss work^{11,12}. Yet the biological mechanism of the disorder is unclear. Prevailing neurobiological models of PTSD focus on the interaction between ventromedial prefrontal cortex (vmPFC), amygdala, and hippocampus^{5,6}. The role of the amygdala in fear and anxiety is well documented¹³, as is the role of hippocampus in episodic memory retrieval¹⁴. vmPFC projects directly to amygdala^{15,16}, and is thought to provide inhibitory input that regulates emotion¹⁷. PTSD patients have reduced hippocampus and vmPFC volumes⁵⁻⁷. When exposed to reminders of traumatic events, PTSD patients exhibit diminished hemodynamic responses in vmPFC²⁻⁴, but exaggerated hemodynamic responses in amygdala^{6,8-10}. Taken together, these data suggest that PTSD is associated with overactivation of the amygdala due to a lack of inhibitory control by vmPFC, as well as deficient hippocampal function. However, imaging data cannot determine whether any of these neuroanatomical findings reflect an underlying cause of the disorder (such as a preexisting risk factor for the development of PTSD or trauma-induced neuropathology that engenders PTSD symptoms), or a secondary effect of the disorder (such as an artifact of primary dysfunction in other brain areas or the neural response to the experience of PTSD symptoms). Lesion studies could, in principle, elucidate the causal contribution of vmPFC, amygdala, and hippocampus by determining if damage to these brain areas changes the likelihood of developing PTSD. However, in an illness such as PTSD that is not amenable to animal lesion studies, this requires the standardized clinical evaluation of a large group of people who suffered the unlikely coincidence of a localizable focal brain lesion as well as emotionally traumatic events. In addition, the lesions would need to adequately sample various areas of the brain, including vmPFC, amygdala, and hippocampus. Remarkably, we have this unique resource available in the Vietnam Head Injury Study (VHIS).

The VHIS (Phase 3) includes 193 Vietnam veterans with lesions distributed throughout the brain as a result of penetrating head injuries sustained during combat and 52 veterans with combat exposure, but no brain injury. Each of these 245 individuals

was evaluated for PTSD using the Structured Clinical Interview for DSM-IV-TR Axis I disorders, Non-Patient edition (SCID-N/P)¹⁸. The structured interview was performed between April 2003 and November 2006 by a psychiatrist trained to administer the SCID-N/P. Veterans were classified as either having developed PTSD at some point in their lifetime (PTSD positive) or having never developed PTSD (PTSD negative). To identify the neural substrates of PTSD, we employed two complementary analyses: 1) an exploratory approach in which we grouped brain-injured veterans according to PTSD diagnosis (positive or negative) and then compared the distributions of lesions between groups, and 2) a hypothesis-driven approach in which we grouped veterans based on lesion location (involvement of vmPFC, amygdala, or neither) and then compared the prevalence of PTSD between groups.

In the exploratory analysis, comparison of the distribution of lesions in the PTSD-positive (n=62) and PTSD-negative (n=131) groups generated a lesion difference map (Fig. 1) that indicates, for each voxel, the difference between the number of veterans with damage to that voxel that did and did not develop PTSD. For example, if 10 veterans have damage to a particular voxel, and 1 of the 10 veterans developed PTSD but 9 of the 10 did not, then that voxel has a value of 1 minus 9, or -8. Thus, large negative values indicate areas where damage is infrequently associated with the development PTSD, whereas more positive values indicate areas where damage is more frequently associated with the development PTSD. This analysis allows us to identify, without any hypothesis, areas of the brain that are important for the development of PTSD. The lesion difference map (Fig. 1) revealed two regions with particularly dense clusters of negatively-valued voxels (areas where damage is associated with a relatively small likelihood of developing PTSD): a frontal region that includes vmPFC bilaterally and an anterior temporal region that includes the amygdala bilaterally. The density of negative-valued voxels was much greater around the amygdala than in more posterior areas of temporal lobe, which contain hippocampus, but not amygdala. This analysis suggests that vmPFC and amygdala are critically involved in the development of PTSD.

To test these hypotheses directly, we divided the VHIS subjects into four groups based on lesion location: 1) significant damage to vmPFC in either hemisphere (vmPFC lesion group; $n=40$; Fig. 2), 2) damage to amygdala in either hemisphere (amygdala lesion group; $n=15$; Fig. 3), 3) damage not involving vmPFC or amygdala (non-vmPFC/non-amygdala lesion group; $n=133$), and 4) no brain damage (non-brain damaged group; $n=52$). The groups were similar on demographic variables, basic cognitive function, and aspects of military service, including combat exposure (Supplementary Tables 1 and 2). The proportion of veterans diagnosed with PTSD in each group is shown in Table 1. Nearly half (48%) of veterans in the non-brain damaged group developed PTSD. There was a similar PTSD prevalence in the non-vmPFC/non-amygdala lesion group (40%; $p=.31$). These PTSD prevalences are comparable to published estimates of PTSD prevalence among Vietnam veterans exposed to intense combat¹⁹. By contrast, only 18% of the vmPFC lesion group developed PTSD. The prevalence of PTSD in the vmPFC group was significantly lower than in the non-brain damaged group ($p=.002$) and the non-vmPFC/non-amygdala lesion group ($p=.009$). PTSD prevalence was even lower in the veterans with amygdala damage—none of whom ever developed PTSD. The prevalence of PTSD in the amygdala group (0%) was significantly lower than in the non-brain damaged group ($p=.0005$) and the non-vmPFC/non-amygdala lesion group ($p=.001$). The difference in PTSD prevalence between vmPFC and amygdala groups was non-significant ($p=.09$).

It is possible that the absence of PTSD in the amygdala group was due to accompanying damage in anterior temporal cortex or medial temporal lobe structures, rather than damage to the amygdala per se. To address this possibility we selected the veterans who had anterior temporal and/or medial temporal lobe damage, but no amygdala damage ($n=28$). The proportion of veterans in this group who developed PTSD (32%) was significantly greater than in the amygdala group ($p=.01$), but not significantly different than in the rest of the non-vmPFC/non-amygdala group ($p=.38$) or the non-brain damaged group ($p=.17$). In a more narrowly focused analysis, we considered specifically whether hippocampus damage could account for the absence of PTSD in the

amygdala group. Between the amygdala group and the temporal lobe comparison group, twenty individuals had damage involving hippocampus (11 in the amygdala group, 9 in the temporal lobe comparison group). Of the nine veterans with hippocampus damage but intact amygdala, four (44%) were diagnosed with PTSD. This proportion is similar to the PTSD prevalences in the non-vmPFC/non-amygdala lesion group (40%) and non-brain damaged group (48%), but significantly higher than the PTSD prevalence in the group with damage to both hippocampus and amygdala (0%; $p=.03$). Furthermore, basic memory encoding and retrieval functions were intact in the amygdala group (Supplementary Table 4). These data support the conclusion that damage to the amygdala, rather than hippocampus or other temporal areas, is the basis of the amygdala group's conspicuous lack of PTSD.

In summary, veterans with vmPFC or amygdala damage were significantly less likely to develop PTSD than veterans with damage to other parts of the brain, or veterans with no brain damage. Particularly striking was the complete absence of a lifetime diagnosis of PTSD among veterans with amygdala damage, which could not be attributed to damage to surrounding temporal lobe regions, including hippocampus.

We further investigated whether the reduction in PTSD following vmPFC or amygdala damage was specific to PTSD, or if it applied to anxiety disorders in general. VHIS subjects were evaluated with the SCID-N/P for panic disorder, agoraphobia, social phobia, specific phobia, obsessive compulsive disorder, generalized anxiety disorder, substance-induced anxiety disorder, and anxiety disorder not-otherwise-specified. The proportion of individuals diagnosed with any of these anxiety disorders was not significantly different among the amygdala group (13%), vmPFC group (15%), and the non-vmPFC /non-amygdala lesion group (22%) ($p>.10$). Although the overall prevalence of non-PTSD anxiety disorders was lower than that of PTSD (meaning less power to detect differences between groups), these data nonetheless suggest that vmPFC and amygdala play a particularly critical role in PTSD, rather than in anxiety disorders in general.

These results from a unique sample of brain-damaged and trauma-exposed individuals provide evidence that vmPFC and amygdala are causally involved in the pathogenesis of PTSD. In both brain areas, unilateral damage appeared sufficient to reduce the occurrence of PTSD. Conventional neurobiological models propose that deficient modulation of amygdala by vmPFC and hippocampus is the underlying mechanism of the disorder^{5,6}. We found no evidence that hippocampus damage affected the development of PTSD. The finding that amygdala damage eliminated the occurrence of PTSD supports one aspect of the model, i.e., that amygdala hyperactivity is a critical element. However, the finding that vmPFC damage independently reduced the occurrence of PTSD argues against the theory that decreased vmPFC inhibition is the basis of the amygdala hyperactivity. If a loss of vmPFC inhibition of the amygdala were the neuroanatomical basis of PTSD, then one would expect vmPFC damage to increase the occurrence of PTSD. The fact that vmPFC damage decreased the occurrence of PTSD indicates that vmPFC has a role in the expression of PTSD—perhaps vmPFC’s interaction with the amygdala is modulatory, rather than simply inhibitory. It has been proposed that vmPFC is critical for the re-activation of emotional states associated with past experiences^{20,21}. Our results and observations²² are consistent with this account of vmPFC function. Moreover, our results indicate that damage to vmPFC or amygdala can protect against the pathological re-activation of traumatic memories central to PTSD. These findings suggest that treatments aimed at selectively disrupting vmPFC and/or amygdala function²³⁻²⁸ could have efficacy to treat PTSD.

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Table 1. PTSD prevalence.

Brain damage	PTSD prevalence
Amygdala	0%
vmPFC	18%
non-vmPFC/non-Amygdala	40%
No brain damage	48%

A chi-square frequency analysis of the three groups of brain-injured veterans indicates a significant effect of lesion location on PTSD prevalence ($\chi^2=14.7$; $p=.0006$). p-values for individual pairwise comparisons are summarized in Supplementary Table 3. Chi-square tests are used for pairwise comparisons if there are at least five individuals with (or without) PTSD diagnosis in both groups; if not, Fisher's exact test is used.

Figure 1. Lesion difference analysis. A healthy adult brain is shown on the left. The lesion difference analysis for the corresponding slices is shown on the right. The color of each voxel indicates the difference between the number of veterans with damage to that voxel that developed PTSD and the number of veterans with damage to that voxel that did not develop PTSD. The colors blue and green indicate the most negative values—areas where damage is relatively infrequently associated with PTSD. Top row: Sagittal views of negative value clusters in prefrontal cortex. The left hemisphere ($x=-10$) is on the left; the right hemisphere ($x=16$) is on the right. Second row: Coronal views of negative value clusters in bilateral prefrontal cortex. Slices are arranged with the anterior-most slice on the left ($y=66$; $y=56$; $y=46$; $y=36$; respectively). Third row: Coronal views of negative value clusters in bilateral anterior temporal lobe ($y=14$; $y=8$; $y=2$; $y=-4$; respectively). Fourth row: Coronal views of posterior temporal lobe ($y=-10$; $y=-16$; $y=-22$; $y=-28$; respectively).

Figure 2. vmPFC group lesion overlap map. The color indicates the number of veterans in the vmPFC group ($n=40$) with damage to a given voxel. The greatest lesion overlap (red) occurs in the anterior vmPFC bilaterally (BA 10/11). Top row: Sagittal views of the vmPFC group lesion overlap. The left hemisphere ($x=-8$) is on the left; the right hemisphere ($x=6$) is on the right. Middle row: Coronal views of a healthy adult brain. Slices are arranged with the anterior-most slice on the left ($y=66$; $y=56$; $y=46$; $y=36$; respectively). Bottom row: Coronal views of the vmPFC group lesion overlap, corresponding to the slices in the middle row. Of the forty vmPFC patients, fourteen had bilateral vmPFC lesions, fifteen had exclusively or predominantly left vmPFC lesions, and eleven had exclusively or predominantly right vmPFC lesions. Of the seven vmPFC patients who developed PTSD, two had bilateral vmPFC lesions, two had exclusively or predominantly left vmPFC lesions, and three had exclusively or predominantly right vmPFC lesions.

Figure 3. Lesion overlap maps for the amygdala and temporal lobe

comparison groups. The color indicates the number of veterans with damage to a given voxel. Top row: Coronal views of a healthy adult brain. Slices are arranged with the anterior-most slice on the left ($y=14$; $y=8$; $y=2$; $y=-4$; respectively). Middle row: Coronal views of the amygdala group lesion overlap. Bottom row: Coronal views of the temporal lobe comparison group lesion overlap. Slices in the middle and bottom rows correspond to the top row. The overlap maps are similar, except for the medial anterior temporal area containing the amygdala, which is damaged in the amygdala group but intact in the temporal lobe comparison group. Of the fifteen amygdala patients, seven had damage to the left amygdala and eight had damage to the right amygdala.

Supplementary Methods

Subjects. Subjects were drawn from the W.F. Caveness Vietnam Head Injury Study (VHIS) registry, which includes 1,221 American soldiers who survived penetrating brain wounds suffered in Vietnam. The VHIS has been organized in three phases. Phase 1 was the initial enrollment, which occurred between 1967 and 1970. Phase 2 was conducted approximately 15 years later. For Phase 2 the 1,118 veterans still alive were invited to participate in an extensive follow-up clinical study at Walter Reed Army Medical Center between August 1981 and August 1984. Of the 1,118 survivors, 520 participated in the Phase 2 study. Injury and preinjury characteristics of the soldiers on the original registry were available from military and VA records. Comparison subjects (n=85) were recruited from VA files of non-head-injured soldiers who had served in Vietnam the same years and were within the same age range as soldiers on the Caveness registry. 192 head-injured and 52 non-head-injured subjects from Phase 2 participated in Phase 3, which included a psychiatric evaluation by a neuropsychiatrist (V.R.). Phase 3 was conducted between April 2003 and November 2006 at Bethesda National Naval Medical Center.

Characteristics of each participant group for this study are presented in Supplementary Tables 1 and 2. Groups did not significantly differ from each other on basic demographic variables (age, race, sex, and education), nor did they differ in the age at which they arrived in Vietnam or pre-combat scores on the Armed Forces Qualifying Test (AFQT; a measure of basic intellectual function). As one would expect, AFQT change and combat exposure was greater for the brain-injured veterans than for the veterans without brain damage. Within the brain-injured veterans there was no significant difference in combat exposure among the three groups (amygdala, vmPFC, and non-vmPFC/non-amygdala), both in terms of mean ratings ($F=2.06$; $p=.13$) and proportion of individuals with high exposure ($\chi^2 = 3.40$; $p=0.18$), nor were there any significant differences in AFQT change among the three groups ($F=0.84$; $p=.43$). Thus differences in combat exposure or intellectual decline cannot account for the differences in PTSD occurrence. In fact, the amygdala group had the highest combat exposure but lowest PTSD prevalence.

Lesion Analysis. CT data were acquired during the Phase 3 testing period. Lesion location and volume was determined from CT images using the Analysis of Brain Lesion (ABLE) software^{29,30} contained in MEDx v3.44 (Medical Numerics, Germantown, MD) with enhancements to support the Automated Anatomical Labeling (AAL) atlas³¹. For the hypotheses about specific brain areas (vmPFC and amygdala), regions of interest (ROIs) were defined in terms of AAL structures³¹ and Talairach coordinates³². As part of this process, the CT image of each subject's brain was spatially normalized to a CT Template brain image in MNI space³³. By analyzing the overlap of the spatially normalized lesion image with the AAL atlas image, the percentage of AAL structures intersected by the lesion was determined. Lesion volume was calculated by manual tracing of the lesion in all relevant slices of the CT image then summing the traced areas and multiplying by slice thickness. The manual tracing was performed by a trained neuropsychiatrist (V.R.) and reviewed by J.G., who was blind to the results of the clinical evaluation and neuropsychological testing.

The vmPFC ROI included portions of the following AAL structures: Superior frontal gyrus, medial; Superior frontal gyrus, orbital part; Superior frontal gyrus, medial orbital; Middle frontal gyrus, orbital part; Inferior frontal gyrus, orbital part; Gyrus Rectus; Olfactory cortex; Anterior cingulate and paracingulate gyri. The portions of these structures included in the vmPFC ROI were those areas inferior to the anterior commissure (z value less than zero) and between 0 and 20 mm left and right from the anterior commissure for the left vmPFC (x value between -20 and 0) and right vmPFC (x value between 0 and 20), respectively. These criteria outline an area comprising the ventral portion of the medial prefrontal cortex (below the level of the genu of the corpus callosum) and medial portion of the orbital surface (approximately the medial one-third of the orbitofrontal cortex in each hemisphere) as well as the subjacent white matter.

A subject was included in the vmPFC group if his lesion occupied at least 15% of the right or left vmPFC ROI. We used 15% damage as a threshold for inclusion in the vmPFC group because it has been demonstrated that damage to approximately 15% of the vmPFC in one hemisphere can be sufficient to yield clear impairments in emotional

processing³⁴. Since the amygdala and hippocampus are pre-defined in the AAL atlas it was not necessary to specify criteria for those structures. A subject was included in the amygdala group if his lesion involved any portion of the amygdala in either hemisphere. The amygdala is a much smaller and discrete area than vmPFC, and there is no evidence to suggest a threshold for the effect of partial damage, so any damage to amygdala was presumed to be potentially significant.

Supplementary Data

In order to receive a PTSD diagnosis, the patient must have symptoms in each of three categories: re-experience, avoidance/numbing, and hyperarousal. In a follow-up analysis, we sought to determine the effect of vmPFC and amygdala damage on these specific categories of PTSD symptoms. The Clinician-Administered PTSD Scale-Diagnostic Version³⁵ (CAPS-Dx) was used to assess the number of symptoms present in each of these three domains. We ran a 3 x 4 (“symptom type” x “lesion group”) ANOVA (Supplementary Table 5). As expected we found a significant main effect of “lesion group” on the number of PTSD symptoms ($F=9.87$; $p<.0001$), with the amygdala and vmPFC groups exhibiting fewer overall symptoms than comparison groups. However, there was no significant interaction between “lesion group” and “symptom type” ($F=0.27$; $p>.95$), indicating that damage to vmPFC or amygdala does not selectively diminish individual categories of PTSD symptoms, but rather, reduces symptoms in all three categories to a similar extent.

The CAPS-Dx also includes an assessment of overall lifetime distress associated with PTSD symptoms (rating on a scale of 0-3). As expected based on the diagnosis differences reported in the main text, the mean ratings for distress were greater in the non-brain damaged and non-vmPFC/non-amygdala groups (1.79 and 1.64, respectively) than in the vmPFC and amygdala groups (1.28 and 0.73, respectively).

We also examined the proportion of individuals in each group who were diagnosed with “current PTSD” at the time of the Phase 3 SCID-I/P evaluation. Veterans with “current PTSD” diagnosis are a subset of veterans with “lifetime PTSD” diagnosis. The

proportions of veterans with “current PTSD” diagnosis are as follows: non-brain damaged group (29%); Non-vmPFC/non-amygdala group (18%); vmPFC group (8%); amygdala group (0%). Of the seven vmPFC patients with a lifetime PTSD diagnosis, three (43%) had a current PTSD diagnosis. This proportion (number with current PTSD divided by number with lifetime PTSD) is similar to the non-brain damaged group (60%) and non-vmPFC/non-amygdala group (45%). While the sample size is much too small to make any definite conclusions, these data suggest that vmPFC damage does not affect the longevity of PTSD, if the symptoms are in fact present.

Supplementary Tables

Supplementary Table 1. Participant data at time of Phase 3 clinical evaluation.

Group	n	Age	Sex (% male)	Race (% Cauc)	Education (years)	MMSE
Amygdala	15	57.8 (2.3)	100	93	14.6 (3.2)	27.8 (2.0)
vmPFC	40	58.0 (3.1)	100	92	14.1 (6.7)	27.9 (2.9)
Non-vmPFC/non-amygdala	132	58.5 (3.2)	100	89	15.0 (2.5)	28.6 (1.7)
No brain damage	52	59.0 (2.5)	100	87	15.2 (2.5)	29.1 (1.3)

For “Age,” “Education,” and “MMSE,” mean values are given with standard deviations in parentheses. “Age” refers to years at the time of SCID administration. “Sex (% male)” is the percentage of male subjects. “Race (% Cauc)” is the percentage of Caucasian subjects. “MMSE” is the Mini Mental State Examination³⁶, a test of basic cognitive function. Any score over 25 (out of 30) is effectively normal.

Supplementary Table 2. Military service data.

Group	Age in Vietnam	% Drafted	AFQT (%ile)	AFQT Change	Combat Exposure	Combat Exposure (% high)
Amygdala	20.4 (2.2)	50	65.6 (28.1)	-15.2 (23.9)	3.9 (1.6)	64
vmPFC	20.3 (3.1)	36	55.0 (22.6)	-9.7 (17.9)	3.2 (0.8)	43
Non-vmPFC/ non-amygdala	20.6 (2.9)	31	61.3 (25.9)	-8.2 (19.0)	3.3 (1.1)	58
No brain damage	20.6 (3.1)	52	65.4 (22.9)	3.9 (14.5)	2.6 (1.2)	33

“Age in Vietnam” is the age when arrived in Vietnam; “% Drafted” is the percentage of individuals drafted into military service; AFQT (Armed Forces Qualifying Test) is a measure of basic intellectual function; “AFQT (%ile)” is the AFQT percentile at the time of enlistment; “AFQT Change” is the difference between AFQT score at the time of enlistment and the Phase 3 evaluation; “Combat Exposure” is the frequency of exposure to enemy contact: 0=no contact (support unit); 1=occasional mortar attack (support unit); 2=intermittent enemy contact (support unit); 3=intermittent enemy contact (combat unit); 4=constant enemy contact (combat unit). “Combat Exposure (% high)” is the percentage of individuals who experienced constant enemy contact in a combat unit. For “Age in Vietnam,” “AFQT (%ile),” “AFQT change,” and “Combat Exposure” mean values are given with standard deviations in parentheses.

Supplementary Table 3. Pairwise comparisons of lifetime PTSD prevalence.

Pairwise comparison	p-value
Amygdala vs. No brain damage	0.0005
Amygdala vs. Non-vmPFC/non-amygdala	0.001
vmPFC vs. No brain damage	0.002
vmPFC vs. Non-vmPFC/non-amygdala	0.009
Amygdala vs. Non-amygdala temporal	0.01
vmPFC vs. Amygdala	0.09
Non-amygdala temporal vs. No brain damage	0.17
Non-vmPFC/non-amygdala vs. No brain damage	0.31
Non-amygdala temporal vs. Non-vmPFC/non-amygdala	0.45

Significant differences ($p < .05$) are in bold. Chi-square tests are used for pairwise comparisons if there are at least five individuals with (or without) PTSD diagnosis in both groups; if not, Fisher's exact test is used.

Supplementary Table 4. Memory function of the amygdala group.

WMS-III Test	Score
General Memory Index	97.2 (17.3)
Auditory Delayed	100.2 (16.4)
Visual Delayed	94.6 (19.0)
Verbal Paired Associates-Recall	16.5 (9.3)

Tests are from the Wechsler Memory Scale-III³⁷. For all tests, group means are given with standard deviations in parentheses. There is no systematic memory impairment in the amygdala group.

Supplementary Table 5. Mean number of PTSD symptoms within each symptom category for each group of VHIS patients.

Group	Reexperience	Avoidance/Numbing	Hyperarousal
Amygdala	1.40	1.07	1.13
vmPFC	2.08	1.87	1.92
Non-vmPFC/Non-amygdala	2.40	2.29	2.16
No brain damage	2.63	2.77	2.31

A 3 x 4 (“symptom type” x “lesion group”) ANOVA reveals a significant main effect of “lesion group” but no interaction effects.

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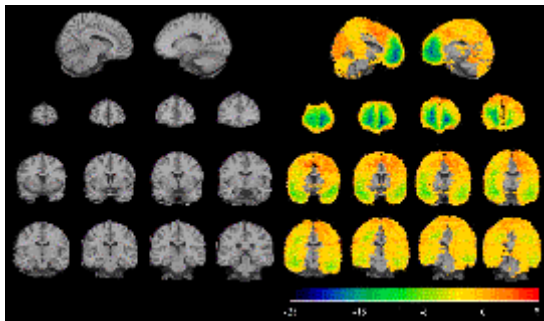


Figure 1

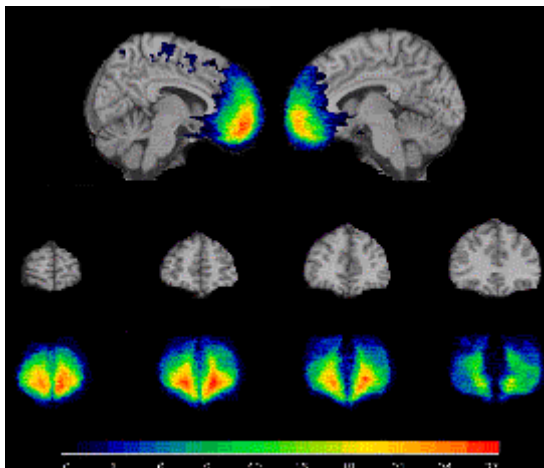


Figure 2

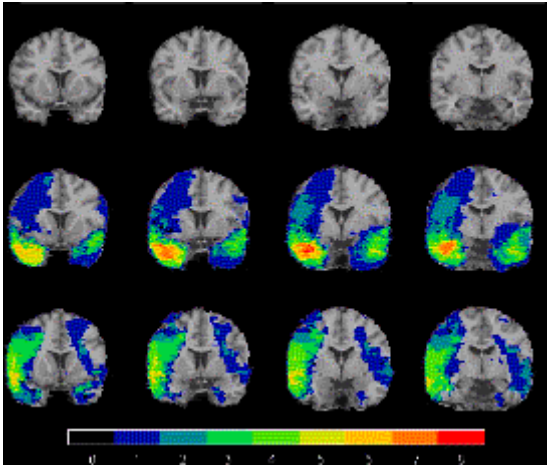


Figure 3